ORIGINAL ARTICLE

A STUDY OF DIRECT AND CONCENTRATED SMEAR MICROSCOPY BY ZEIHL NEELSEN AND FLUORESCENT STAINING FOR DIAGNOSIS OF SUSPECTED TUBERCULOSIS IN TERTIARY CARE HOSPITAL

Rachana Patel[1], Pragnesh Bhuva[2], Mannu Jain[3], Shashwati Bhuva[4], Pinal Mangukiya[5]

Author's Affiliations: [1]Resident; [2]Assi. Professor; [3]Professor and Head; [4]Tutor; [5]Msc MLT student, Dept of Microbiology, SMIMER, Surat, Gujarat.
Correspondence: Dr Rachana Dhirubhai Patel Email: rachana.roze@gmail.com

ABSTRACT

Introduction: For early diagnosis of TB, it is essential to ensure proper identification. Smear microscopy is a simple, economical, less time-consuming technique is a good alternative. The stud was conducted to compare direct smear, concentrated smear and fluroscent microscopy of sputum of patient with tuberculosis; also to assess the sensitivity and specificity of direct and concentrated smear by ZN stain and fluorescent microscopy.

Methodology: The 400 samples of suspected to be a case of pulmonary tuberculosis as per RNTCP guidelines are included. Direct smear and concentrated smear were made and stained by Carbolfuchsin methods which include the Ziehl-Neelsen (ZN) and Kenyon methods (Light /bright field microscope) and Fluorochrome procedure using auramine-O or auramine-rhodamine dyes (Fluorescent microscope -FM).

Result: On direct smear, out of 400 samples 138 samples were positive by ZN stain method and 150 samples were positive by FM stain method. While it was 154 samples and 156 sample by respective stains on concentrated smear. The sensitivity of direct and concentrated smear microscopy is comparable to FM stain and Zn stain. The difference between sensitivities (89.61 versus 96.15%.p<.01) obtained by the two methods was significant. The difference between sensitivities (92% versus 98.71%; p<.01) obtained by the two methods was significant. The specificity (100%) was similar for both techniques.

Conclusion: The study showed that concentrated AFB microscopy is more efficient to detect M. tuberculosis in respiratory specimens than direct AFB microscopy. Fluorescent microscopy has higher sensitivity and comparable specificity which is further enhanced by concentration.

Key words: Direct and concentrated smear microscopy, Zeihl Neelsen and Fluorescent staining, Tuberculosis

INTRODUCTION

India is the country with the highest burden of TB.[1] Early diagnosis of TB is crucial both clinically and epidemiologically. It is essential to ensure proper and early identification of cases and good treatment outcomes to be able to limit its transmission and obtain successful TB control. Smear microscopy is a simple, economical, less time-consuming technique used for early detection, and it also has prognostic value.,This method is specific, faster and cheaper for the detection of acid-fast bacilli (AFB) in sputum. It has suggested that the performance of sputum smear microscopy can be significantly improved if sputum is liquefied with chemical reagents and then concentrated by centrifugation or sedimentation prior to acid-fast staining. The newer alternative technique to Z-N smear microscopy, FM is known to increase the sensitivity (10% higher) when compared with Z-N microscopy methods while speeding up the whole process to consume much lesser time. In this study we wanted to compare the sensitivity and specificity obtained with smears for detection of AFB prepared directly from respiratory specimens (direct AFB smears) to that obtained with the parallel smears prepared from concentrate of the specimens (concentrated AFB smears) and also to compare direct smear, concentrated smear and fluorescent and Zeihl Neelsen microscopy of sputum of patient with tuberculosis.

METHODOLOGY

The present study is hospital based cross sectional observational study conducted over patients attend-

ing RNTCP in the SMIMER. Study was conducted from 11th March 2016 to 31st May 2016. The study group consisted old and new cases of pulmonary tuberculosis minimum total number of cases were 250. Sample size counted as per statistical formula.

A suspect was defined as an individual if he/she had persistent cough for more than three weeks, and/or evening rise of temperature for more than two weeks, and/or body mass index (BMI) less than 16. The sample of all adults of both the gender suspected to be a case of pulmonary tuberculosis as per RNTCP guidelines are included. Samples other than sputum, samples macroscopically resembling saliva are excluded. A total of 400 sputum specimens were aseptically collected and were transported in specimen transportation box (cool box) to the Tuberculosis Laboratory at clinical laboratory for AFB microscopy and culture. According to inclusion and exclusion criteria, 250 suspected/confirmed cases of pulmonary tuberculosis were taken. All they were subjected to a thorough history, clinical examination and laboratory investigations. After sample collection homogenisation and decontamination was done by N-Acetyl L-Cysteine Sodium Hydroxide (NALC-NaOH) method. In cases where there is no spontaneous sputum production, a sample can be induced, usually by nebulized inhalation of a saline or saline with bronchodilator solution.

Here two procedures used for acid-fast staining:[1]Carbolfuchsin methods which include the Ziehl-Neelsen and Kenyon methods (Light /bright field microscope) [2]Fluorochrome procedure using auramine-O or auramine-rhodamine dyes (Fluorescent microscope).

Statistical analysis was performed with IBM SPSS Statistics version 21 Software. Sensitivity and specificity calculated.p-value less than or equal to 0.05 was considered as significance.

RESULTS

In the present study, total 250 cases were taken. Out of that 133(53 %) were new cases and 117(47%) were already diagnosed cases. Out of total of 250 patients, 162(64.8%) patients were male while rest 88(35.2%) patients were female. Majority of patients (80) belong to in age group of 21-30 and minimum numbers of patients (5) in age group of greater then 80 years. Maximum new cases (50) found in age group of 21-30 years and maximum old cases (32) found in age group of 31-40years.

In present study, we found that 16 cases were negative on direct smear came positive in concentration method. Total out of 400, 138 sample were positive by direct smear method and 154 samples were positive for tuberculosis by concentration method. By FM stain 6 samples negative for tuberculosis by direct smear method came out to be positive by concentration method. Total out of 400, 150 samples were positive by direct smear method and 156 samples were positive for tuberculosis by concentration method.

Table 1: Sputum result of study subjects

	1 +	2 +	3 +	SCANTY	NEGATIVE	TOTAL
Direct ZN	76	28	11	23	262	400
Conc. ZN	39	63	37	15	246	400
Direct FM	54	58	17	21	250	400
Conc. FM	17	66	67	6	244	400

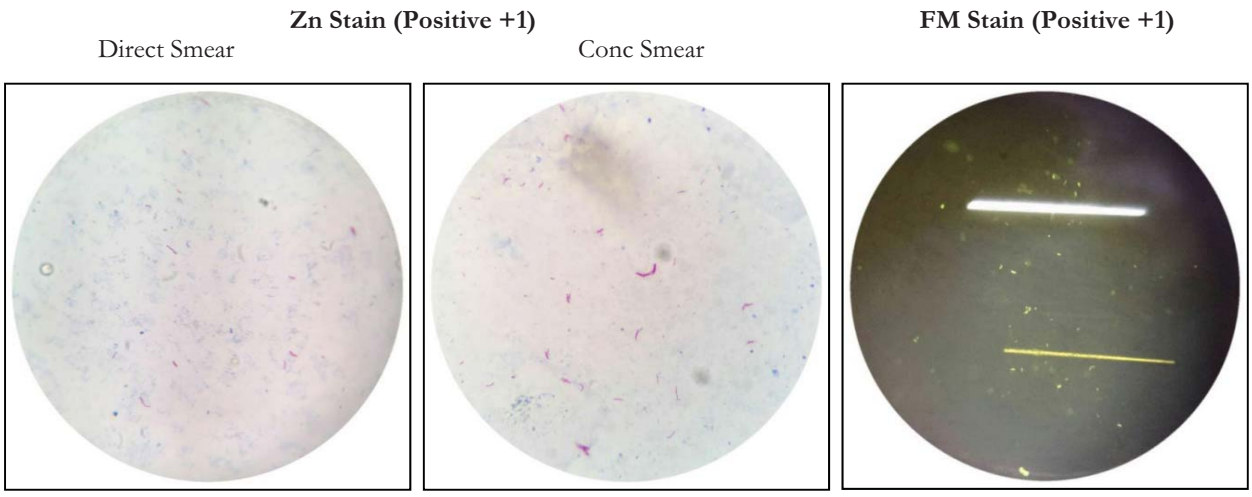

Figure 1: Stains

The sensitivity of direct and concentrated smear microscopy is comparable to FM stain and Zn stain. The difference between sensitivities (89.61 versus 96.15%.p<.01) obtained by the two methods was found to be significant. These results showed that concentrated technique increases the sensitivity of microscopy up to when performed with the same specimens. The specificity (100%) was similar for both techniques.

Table 2: Cross tabulation of Positive smear grades by direct and concentration method by ZN stain

Direct smear results	Negative	Scanty	1+	2+	3+	Total
Negative	246	14	2	0	0	262
Scanty	0	1	21	1	0	23
1+	0	0	16	54	6	76
2+	0	0	0	8	20	28
3+	0	0	0	0	11	11
Total	246	12	39	63	37	400

Table 3: Cross tabulation of Positive smear grades by direct and concentration method by FM stain

Direct smear results	Negative	Scanty	1+	2+	3+	Total
Negative	244	6	0	0	0	250
Scanty	0	0	17	4	0	21
1+	0	0	0	48	6	54
2+	0	0	0	13	45	58
3+	0	0	0	0	17	17
Total	244	6	17	66	67	400

Table 4: Direct FL vs conc. ZN

Direct FL Smear	Conc. ZN Smear		Total
	Positive	Negative	
Positive	150	0	150
Negative	4	246	250
Total	154	246	400

Table 5: Conc. FL vs Direct ZN

Conc. FL Smear	Direct ZN Smear		Total
	Positive	Negative	
Positive	138	18	156
Negative	0	244	244
Total	**138**	**262**	**400**

In present study, on direct smear, we found that out of 400 samples 138 samples were positive by ZN stain method and 150 samples were positive by FM stain method. On concentrated smear, 154 samples were positive by ZN stain method and 156 samples were positive by FM stain method.

The sensitivity of ZN stain and FM stain was different. The difference between sensitivities (92% versus 98.71%; p<.01) obtained by the two methods was found to be significant. These results showed that FM stain increases the sensitivity of microscopy up to 6.71% when performed with the same specimens. The specificity (100%) was similar for both techniques.

DISCUSSION

Early diagnosis of TB is crucial both clinically and epidemiologically. It is essential to ensure proper and early identification of cases, and good treatment outcomes to be able to limit its transmission and obtain successful TB control. AFB microscopy is believed to be the most practical and fastest technique in establishing a diagnosis of pulmonary TB. Concentration method by NALC has been found to increase the sensitivity of microscopy. The newer alternative technique to ZN smear microscopy, FM is known to increase the sensitivity (10% higher) when compared with Z-N microscopy methods while speeding up the whole process to consume much lesser time. Fluorescent AFB can be seen at lower magnification than ZN stained AFB.

Comparison of direct and concentration method: In present study,we found that 16 cases were negative on direct smear came positive in concentration method. Total out of 400, 138 sample were positive by direct smear method and 154 samples were positive for tuberculosis by concentration method. By FM stain 6 samples negative for tuberculosis by direct smear method came out to be positive by concentration method. total out of 400, 150 sample were positive by direct smear method and 156 samples were positive for tuberculosis by concentration method.

The sensitivity of direct and concentrated smear microscopy comparing to FM stain and ZN stain, the difference between sensitivities (89.61 versus 96.15%.p<.01) obtained by the two methods was found to be significant. These results showed that concentrated technique increases the sensitivity of microscopy up to when performed with the same specimens. The specificity (100%) was similar for both techniques.

A study conducted by Barez et al. [2] showed that the sensitivity was almost similar in both methods as described 81.6% for direct method and 82.7% for the concentrated method. In another study, Cattamanchi et al. [3] failed to find a difference in sensitivity between direct and concentrated sputum smear microscopy, the calculated sensitivity of direct and concentrated smear microscopy was not significantly different (51% vs. 52%).

In study of j.d.Harelimana [4] patients under 15 years old, sputum concentration technique showed a difference comparing to the direct smear microscopy (75% vs. 25%, C.I = 95%, P < 0.05). A study conducted by Apers et al.[5] showed that the sensitivity of direct microscopy was 67.5% and the sensitivity of the concentration method 87.1%, an increase of 29%.

A study by Mindolli et al.,[6] showed that there was a significant increase in the sensitivity with the use of 5% NaOCl . The increase in the 23.14% smear positivity with the use of 5% NaOCl with the centrifugation method was very encouraging as compared to that of the direct smears.

A study by Kaore et al.,[7] showed that there is rise of 7.11% in sputum positivity over direct microscopy by concentration method. A study by Ongkhzmmy et al., reported that the implementation of the bleach method yields an overall increase in positivity of 33.5% .

Comparison of ZN stain and FM stain: In present study, on direct smear ,we found that out of 400 samples 138 samples were positive by ZN stain method. And 150 samples were positive by FM stain method. On concentrated smear, 154 samples were positive by ZN stain method and 156 samples were positive by FM stain method.

The sensitivity of ZN stain and FM stain was different. The difference between sensitivities (92% versus 98.71%; p<.01) obtained by the two methods was found to be significant. These results showed that FM stain increases the sensitivity of microscopy up to 6.71% when performed with the same specimens. The specificity (100%) was similar for both techniques.

In a study of Zaib-un-Nisa, Javed H et al 2015 overall positivity increased by 2/9 (22.22%) by FL microscopy over the conventional ZN method. The difference in case detection was found to be statistically significant (p<0.00). FL technique has a better diagnostic value and is less time-consuming compared to ZN in diagnosing tuberculosis in pediatric patients. FL microscopy had more positive predictive value (PPV) than ZN microscopy.

In a study of Roma Goyal et al[8] a total of 388 clinically diagnosed pulmonary tuberculosis patients were included in the study Out of 388 sputum samples, the smear positivity for AFB on the conventional ZN method was 7.47% (29/388) while the positivity increased to 14.69% (57/388) on the modified fluorescent method.

In study of Saroj et al[9] 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.

Table 6: Comparison of slide positivity rate between ZN and FM in various studies

Study	Slide +ve rate by ZN	Slide +ve rate by FM	Sample size
Prasanthi et al [10]	50%	69%	38
Ulukanligil et al[11]	9.89%	12.47%	465
Golia S et al [12]	10.41%	16.56%	634
Suria et al[13]	12.40%	19.10%	225
Jayachandra et a[14]	9.70%	Not done	196
Our study	34.50%	37.50%	400

In present study, it was clearly evident that FM staining was more sensitive method than ZN staining in both direct and concentrated technique.

CONCLUSION

It was observed that maximum number of pulmonary tuberculosis patients of our study group was in the 21-30 years age group. The sensitivity of direct and concentrated smear microscopy comparing to FM stain and zn stain. The difference between sensitivities (89.61 versus 96.15%.p<.01) obtained by the two methods was found to be significant. Concentration increases the sensitivity of test by 6.54%.

The sensitivity of ZN stain and FM stain was different. The difference between sensitivities (92% versus 98.71%; p<.01) obtained by the two methods was found to be significant. FM stain is more sensitive then ZN stain.FM stain was 6.71% more sensitive then ZN stain.

The study showed that concentrated AFB microscopy is more efficient to detect M. tuberculosis in respiratory specimens than direct AFB microscopy. Fluorescent microscopy has higher sensitivity and comparable specificity which is further enhanced by concentration.

Sputum examination for the tubercle bacilli is usually conducted for patients clinically and/ radiologically suspected of pulmonary tuberculosis. However, the

standard method of sputum examination, that is, ZN staining is not sensitive enough and a large number of the suspected cases miss diagnosis. Moreover many cases remain unsuspected and don't seek treatment.

Fluorescent stain is a more efficient over ZN stain in detecting Tubercle bacilli in sputum. Fluorescence has been found to be less time consuming compared to ZN method in the diagnosis of tuberculosis.

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