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Diagnosis of pulmonary tuberculosis by smear microscopy and culture in a tertiary health care facility

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Abstract

Smear microscopy and culture forms the backbone of tuberculosis (TB) laboratory investigations in tertiary healthcare facilities which have a large number of cases and financial constraints. The present study aimed to re-evaluate the efficiency of smear microscopy and culture on Lowenstein Jensen (LJ) medium for acid fast bacilli (AFB) isolated from patients with pulmonary tuberculosis. 210 samples were processed for detection of AFB by Ziehl Neelsen (ZN) staining. Concentration method of N-acetly-L-cystein-NaOH was used and the samples were isolated on LJ medium. AFB was seen in 168 (80.0%) primary smear samples. The primary smear missed 5 (11.9%) samples that were detected by secondary smear (Sensitivity 93.45%, Specificity 88.10%, Positive predictive value (PPV) 96.91% and Negative Predictive value (NPV) 77.08%). Growth was observed in 155 (83.30%) samples (Sensitivity 95.39%, Specificity 70.59%, PPV 93.55%and NPV 77.42%). The values were statistically significant. The present study reconfirms the efficiency of conventional ZN staining method and culture on LJ medium in the detection of AFB in sputum samples of patients with pulmonary tuberculosis.

Keywords: Acid fast bacilli; culture; Lowenstein Jensen medium; pulmonary tuberculosis; smear microscopy; Ziehl Neelsen staining.

Introduction

Millions of people have succumbed to tuberculosis worldwide. Tuberculosis is out of control in most of the developing countries. Despite all the advances made in tuberculosis control, India accounts for one-third of the cases in Southeast Asia (WHO Report, 2009). The Revised National Tuberculosis Control Program (RNTCP) continues high success rates in the detection of new cases and treatment. During the first quarter of 2011, over 2 million suspected cases were examined, 23.4% sputum positive cases were diagnosed and 36.8% TB cases were registered for treatment (Status Report on RNTCP, 2011). Smear microscopy by ZN staining plays an important role in case detection in RNTCP, while culture on LJ medium is the gold standard. With the emergence of MDR and XDR TB, case detection and identification retains a very high priority. With this study we wish to emphasize the efficiency of ZN staining method and Culture on LJ medium.

Materials and Methods

The study was approved by the local ethical committee and Informed consent was obtained from patients. A total of 210 adult patients suspected to suffer from Pulmonary Tuberculosis on the basis of their clinical and radiological presentations were enrolled. Old cases of Pulmonary Tuberculosis were also included in our study. The clinical criteria and patient history was taken. Children and cases of Extra-pulmonary TB were excluded from the study. Early morning sputum samples from patients suspected to suffer from pulmonary tuberculosis were collected in sterile wide mouth containers and examined. Instructions were given to the patients before sputum collection. Sputum samples were processed immediately after receiving it from the patients. A smear was made on a glass slide and stained by ZN staining technique. The smear was checked for acid-fast bacilli under oil immersion and reported according to the Revised National Tuberculosis Control Program (RNTCP laboratory module – 1999).

Negative = No AFB seen per 100 oil immersion fields.
Scanty = 1–9 AFB per 100 oil immersion fields. Give the exact number.
1+ = 10–99 bacilli per 100 oil immersion fields.
2+ = 1–10 AFB per oil immersion field.
3+ = More than 10 AFB per oil immersion fields.

Sputum samples were decontaminated using N – acetyl – L – cysteine + 2 % NaOH and concentrated by centrifugation at 2500 – 3000 rpm. Samples were processed and inoculated on Lowenstein Jensen medium slants. They were incubated for about 8 to 10 weeks at 37^{o} C and checked for growth every week. Colony characteristics were studied (Kent and Kubica, 1985).

The results and observations were subjected to statistical analysis (Pearson Chi-square test, Continuity Correction, Measurement of Agreement (Kappa) and Likelihood Ratio) wherever necessary. Statistical analysis was done using SPSS software version 13 (SPSS Inc., Chicago, Illinois).

Results

210 patients were enrolled in this study. 185 (88.1 %) patients had a previous history of TB. 168 (80.0 %) samples showed the presence of acid-fast bacilli in the primary smear. 42 (20.0 %) did not show the presence of AFB. 2 + smear grading was most commonly seen followed by 1 + smear grade (Table 1). After decontamination and concentration, there were 48 (22.9 %) smear negative samples.

Table 1: Primary & Secondary Smear among the cases.

	Primary Smear Number (%)	Secondary Smear Number (%)
Negative	42 (20)	48 (22.9)
Positive	168 (80)	162 (77.1)
1+	50 (23.8)	
2+	60 (28.6)	
3+	36 (17.1)	
Scanty	22 (10.5)	
Total	**210 (100)**	

Table 2: Primary Smear result compared with Secondary Smear.

Primary Smear result		Secondary Smear		Total
		Positive	Negative	
Positive	**No.**	157	11	168
	%	93.50%	6.50%	100.00%
Negative	**No.**	5	37	42
	%	11.90%	88.10%	100.00%
Total	**No.**	162	48	210
	%	77.10%	22.90%	100.00%

Chi-square Test applied	Value	df	P-value	Association is-
Pearson Chi-Square	126.720	1	2.14E-29	**Significant**
Continuity Correction	122.137	1	2.15E-28	**Significant**
Likelihood Ratio	113.870	1	1.39E-26	**Significant**

Measurements	Value	95 % Confidence Interval	
Sensitivity	93.45%	88.91%	96.51%
Specificity	88.10%	75.57%	95.50%
Positive Predictive Value	96.91%	92.94%	98.99%
Negative Predictive Value	77.08%	62.73%	87.95%
Likelihood Ratio	7.850		

Symmetric measures among the cases

Measure of Agreement	Value	Asymp. Std. Error	Approx. T	Approx. Sig.
Kappa	0.774	0.054	11.257	2.14E-29

BMID: AR93-BM12

5 (11.9 %) samples that were missed in the primary smear were detected by secondary smear. While 11 (6.50 %) samples that were primary smear positive were missed in secondary smear. The Sensitivity and Specificity was found to be 93.45% and 88.10 % for Primary and secondary smear respectively. The Positive predictive value and Negative Predictive value was 96.91 % and 77.08 %. The values were statistically significant (Table 2).

Growth was observed in 155 (73.8 %) samples (n = 186), Contamination was found in 24 (11.4 %) samples and 31 (14.8%) samples did not show growth on LJ medium. Growth was seen in 145 (95.4 %) smear positive samples while 7 (4.6 %) smear positive samples did not show growth. Growth was observed in 10 (29.4 %) smear negative samples (Sensitivity 95.39 % and Specificity 70.59 %). The Positive predictive value and Negative Predictive value was 93.55 % and 77.42 % respectively. The values were statistically significant (Table 3).

Table 3: Smear results compared with Culture on LJ medium.

Smear result		Culture (LJ) (n = 186)		Total
		Growth	No growth	
Positive	No.	145	7	152
	%	95.40%	4.60%	100.00%
Negative	No.	10	24	34
	%	29.40%	70.60%	100.00%
Total	No.	155	31	186
	%	83.30%	16.70%	100.00%

Chi-square Test applied	Value	Df	P-value	Association is-
Pearson Chi-Square	87.098	1	1.03E-20	Significant
Continuity Correction	82.412	1	1.11E-19	Significant
Likelihood Ratio	69.650	1	7.08E-17	Significant

Measurements	Value	95 % Confidence Interval	
Sensitivity	95.39%	90.75%	98.13%
Specificity	70.59%	52.52%	84.88%
Positive Predictive Value	93.55%	88.44%	96.86%
Negative Predictive Value	77.42%	58.94%	90.40%
Likelihood Ratio	3.243		

Discussion

The menace of Tuberculosis is age old. Concerns are mounting over the rise in the number of TB cases and more worryingly, drug resistant TB cases. Despite the fact that highly effective drugs are available today, the problem of this infectious disease is increasing. This situation has been worsened by factors such as delayed diagnosis, high mortality, deteriorating social conditions, emergence of HIV / AIDS, and the poor implementation of DOTS (Directly Observed Treatment, Short – course) strategies. Current evidence suggests that the high levels of project management needed for the success of DOTS strategies are not being achieved.

Passive case finding is the mainstay of case finding in most developing countries. This relies heavily on sputum examination by smear and culture, chest radiology and tuberculin skin testing. Under ideal circumstances, a test for tuberculosis should be 100 % sensitive (no false negative) and 100 % specific (no false positive), should distinguish between infection and disease and give a rough idea of bacillary load.

No such test exists and all existing diagnostic methods reflect compromises of the above ideal. Hence periodic evaluation studies of existing diagnostic methods are a continuing necessity.

The acid-fast smear has been used as an aid in the diagnosis of mycobacterial disease for many years. It is the simplest procedure currently available to detect AFB in clinical samples by ZN staining. As seen in Table 1, 80 % of the samples showed the presence of AFB in the primary smear. Most of the primary smears showed 2 + grading (28.6 %) followed by 1 + (23.8 %) and 3 + (17.1 %). After decontamination and concentration by NALC-NaOH method, 77.1 % samples were AFB positive. 11 samples which were primary smear positive were missed in the secondary smear. This can be attributed to the fact that decontamination / concentration could lead to a decrease in AFB numbers. Thus the secondary smear must have been negative. The values were statistically significant. (P = 2.14E-29). There was an 11.9 % increase in AFB positivity in the secondary smear. This can be attributed to the fact that the amount of AFB in the sputum must be very less and only after concentration the AFB could be seen (Table 2).

The sensitivity of smear in our study was found to be 93.45 % and Specificity was 88.10 %. The PPV was 96.91 % and the NPV was 77.08 %. Boyd and Marr (1975) found the Sensitivity to be only 22 %, and Specificity was 100 %, while Burdash *et al.* (1976) found Sensitivity to be 42.7 % and Specificity to be 99.9 %. Rickman and Moyet (1980) studied the co-relation of sensitivity with that of centrifugal force and observed Sensitivity of 82.4 % at an RPM of 3,800 g and Specificity to be 97.4 %. These observations were comparatively similar to ours. An increase in sensitivity can be misleading as it may be accompanied by a decrease in the true positivity, and an increase in the relative number of false positives. This can be explained by the large number of treated patients who provided specimen that were smear positive but culture negative.

Though the growth of mycobacteria is slow, culture remains the 'Gold Standard' for definitive diagnosis of tuberculosis since it gives positive results with comparatively fewer organisms, and hence permits identification and drug susceptibility tests to be done on the culture isolate (Davies *et al.*, 1996). Growth was seen in 73.8 % samples while contamination was found in 11.4 % samples. All the isolates were identified as that of *M. tuberculosis* using standard biochemical tests.

Though stringent aseptic precautions and suitable decontamination techniques were followed, a large number (11.4 %) of the samples were contaminated. Paramsivan *et al.* (1987) reported a contamination rate of 4 %. This contamination could be attributed to the fact that LJ medium, being an extremely rich and notorious medium, supports the growth of a small number of contaminating bacteria. It has been quoted by Jenkins (1994) that 2 – 3 % of culture could be lost due to contamination, indicating that the treatment procedure is neither too harsh nor too moderate.

Growth was observed in 29.4 % smear negative samples thereby indicating the superiority of culture over smear microscopy, whereas no growth was observed in 4.6 % smear positive samples. All these cases were on AKT, indicating that the bacilli seen in the primary smears were killed bacilli. The Sensitivity and Specificity was found to be 95.39 % and 70.59 % respectively (PPV 93.55 % and NPV 77.42%). The observations seen in Table 3 were statistically significant (P = 1.03E-20).

Conclusion

The present study reconfirms the use of conventional ZN staining method and culture on LJ medium in the detection of AFB in sputum samples of patients with pulmonary tuberculosis in tertiary healthcare facilities which have a large number of cases and financial constraints.

Ethical Approval

The study was approved by the institutional ethics committee of TN Medical College and BYL Nair Charitable Hospital, Mumbai, India.

Conflict of Interests

Authors declare that they have no conflicting interests with regard to the study.

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