

Research Article

Study of Same Day Sputum Smear Examination in Diagnosis of Pulmonary Tuberculosis under RNTCP

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Abstract: Tuberculosis (TB) remains a worldwide scourge, infecting one-third of the world's population. In India, more than 40% of population is infected with TB bacilli with many TB cases remaining undetected due to lack of inexpensive, sensitive and robust diagnostic tests. Diagnosis of pulmonary TB relies upon sputum smear microscopy. The standard two-day method contributes to high patient drop-outs. The present study was undertaken to assess if two spot sputum samples collected on a single day at an hour interval were sufficient to detect AFB in TB suspects and compare microscopy results with the standard method based on older and recent RNTCP guidelines (April 2009). A total of 250 TB suspects were screened for AFB according to RNTCP guidelines and an extra sample was collected on day one, an hour after the first sample was given. Case detection rate was found to be 40.4% and 38% with gold standard and one-day strategy respectively based on older guidelines. By recent guidelines, case detection was found to be 40.4% and 39.6% with two-day and one-day method respectively. The diagnostic process could be made much more efficient and convenient for patients if it could be completed in a single day by examining two same-day specimens which was found to be as effective as two-day strategy.

Keywords: Tuberculosis; Diagnosis; Sputum smear microscopy; Standard two-day method; AFB; RNTCP; Case detection rate.

INTRODUCTION

Tuberculosis (TB), the most prevalent disease in the world has infected one-third of the world's population with India accounting for one-fifth of the global incidence with 18 lakh new cases and 4 lakh TB related deaths occurring annually.[1] In India approximately 20 lakh people are estimated to die of TB in the next five years, unless sustained and appropriate action is taken.[1] Thus, World Health Organisation (WHO) has reaffirmed tuberculosis as a continuing global emergency.

Undiagnosed and untreated sputum positive TB cases are the source of infection in the community. Every smear positive person if left untreated has the potential to infect 10-15 persons/year.[1] Under Revised National Tuberculosis Control Program (RNTCP),[2] which aims at achieving 70% case detection rate, diagnosis in developing countries depends on direct smear examination of sputum. Sputum microscopy is the pillar of RNTCP both for diagnosis and follow-up of the patients.[1] In 2004, case detection rate in India was found to be only 53%.[1] Thus, failure to promptly identify and treat infectious cases stands out as a critical obstacle to TB control.

The older RNTCP guidelines required microscopic examination of three sputum specimens for Acid Fast Bacilli (AFB) collected over two days wherein two positive smears were diagnostic.[1] Recent update of RNTCP guidelines (April 2009) recommend collection of two samples over two days including an early morning sample out of which even a single positive sample is diagnostic.[3] This causes an increase in laboratory workload, high rate of patient drop-out during the diagnostic process due to patient inconvenience and in turn low case detection rate.[4] Several studies have shown that two sputum specimens proved adequate in the diagnosis of tuberculosis and the third specimen added little additional diagnostic value.[5,6,7,8]

In RNTCP cell of our institute, (VIMS, Bellary), it was observed that out of 4551 TB suspects, 61.1% provided three sputum samples and 38.9% gave only one sample and failed to return the next day in 2006. Therefore, the present study, was carried out to assess if examination of two sputum samples collected on the same day at an interval of one hour is sufficient

for detecting AFB instead of the three/two samples over two consecutive days under RNTCP.

MATERIALS AND METHODS

The present study was carried out in the Department of Microbiology, Vijayanagar Institute of Medical Sciences, Bellary, over a period of one year from 1st January 2008 to 31st December 2008.

Source of data: Study included sputum samples collected from a total of 250 new adult patients presenting with history of cough and expectoration for three weeks or more attending Medical College Hospital, for clinical diagnosis and treatment.

Exclusion criteria:

- 1) All extra pulmonary tuberculosis cases
- 2) Known Human Immunodeficiency Virus positive cases.

Sample collection: Sputum samples were collected from patients according to RNTCP guidelines.[1,9] An additional sample was collected on day 1, one hour after the first sample as mentioned below. Those who failed to provide the samples the following day were not included in the study.

1st sample (A) - 1st spot sample on day 1.

2nd sample (B) - 2nd spot sample on day 1, one hour after 1st sample was given.

3rd sample (C) - Early morning sample on day 2.

4th sample (D) - 3rd spot sample on day 2.

Laboratory procedures: All the sputum samples received were processed as per RNTCP guidelines.[9] Random blinded rechecking of both positive and negative slides was done by Senior Tuberculosis Laboratory Supervisor (STLS) at District Tuberculosis Centre, Bellary. Evaluation was done by following way: (1) Comparison was done between previous gold standard two-day RNTCP strategy[9] (three samples - wherein 2 positive smears were diagnostic) and one-day test method (two samples collected on first day at an hour interval with 2 smears being diagnostic). (2) Comparison was also made between the current RNTCP strategy[3] (two samples collected over two consecutive days wherein a single positive smear is

diagnostic) and one-day test method (one smear being diagnostic).

Statistical Analysis: - Proportion of positive samples was compared using Chi square test of significance. The sensitivity, specificity, positive and negative predictive values of two and three smears were calculated and compared.

RESULTS

Out of the study group of 250 patients, 197 (79%) were males and 53 (21%) were females. Most of the patients were in the age group between 31-60 years (n=158 - 63.2 %) with mean age of presentation of 42.8 years. The maximum number of chest symptoms fell in the range of 31- 40 years. 70 (28%) were inpatients in VIMS hospital and remaining 180 (72%) were out patients. The commonest risk factors was found to be smoking (32%) followed by contact history (13.2%). But in 45.2% of cases, there was no associated risk factor. Analysis shows an association between risk factors and positivity (p value – 0.005 Chi-square analysis, Odds ratio – 2.07 [95% CI= 1.19 – 3.61]). The commonest presenting symptom was cough with associated symptoms like fever, nausea, headache, weight loss (68.8%). The mean duration of symptoms was found to be 13.2 weeks. (S.D. - 10.3).

In the present study, out of 250 patients, sample 'A' (1st spot) showed 99 (39.6%) to be positive for AFB and 151 (60.4%) to be negative, sample 'B' (2nd spot) was found to be positive in 95 (38%) patients and negative in 155 (62%) patients, sample 'C' (early morning sample) was found to be positive in 101 (40.4%) patients and negative in 149 (59.6%) patients and 100 (40%) patients were found to be positive in sample 'D' only (3rd spot) and negative in 150 (60%) patients. Two cases were found to be sputum negative on day one with both samples A and B, but were found to be smear positive the next day with samples C and D (2- 0.8%). No additional samples were found positive with B and D samples. Sample C (early morning sample) yielded 6 (2.4%) additional positive cases. Sample B failed to detect AFB in 6 (2.4%) cases in toto out of which 4 (1.6%) cases showed AFB in A samples with negative B samples. Sample D failed to recognize 1 (0.4%) case. (TABLE 1)

Table 1: Positivity Rate (Cases Detected Samplewise)

Sputum Samples	A	B	C	D	AB	A,C,D	A,B	A,C
					Old RNTCP guidelines		New RNTCP guidelines	
Total examined	250	250	250	250	-	-	-	-
No. positive	99 39.6%	95 38%	101 40.4%	100 40%	95 38%	101 40.4%	99 39.6%	101 40.4%

Based on old¹ (samples A, C, D) and recent³ (samples A, C) RNTCP guidelines, standard two-day strategy detected all 101 positive cases (100%) with Sensitivity, Specificity, Positive predictive value and Negative predictive value of 100% each. Based on old guidelines, one-day method (samples A and B) detected 95 (94.05%) patients out of total 101 diseased with Sensitivity of 94.05%, Specificity of 100%, Positive predictive value of 100%, and Negative predictive value of 96.12% and Accuracy of 97.6 when compared to two-day strategy. The one-day test method failed to detect 6 cases with false negatives of 5.95%.

Incremental yield of two-day strategy compared to one-day strategy was found to be 5.9%. Statistical analysis showed one-day test approach to be slightly inferior to two day method in detecting positive cases among diseased (p value = 0.01). According to current RNTCP guidelines, the one-day test strategy (samples A and B) detected 99 (98.01%) AFB positive cases with Sensitivity of 98.01%, Specificity of 100%, Positive predictive value of 100%, Negative predictive value of 98.67%, Accuracy of 99 and false negatives of 1.98%. Both methods were found to be almost similar in detecting cases after statistical analysis (p = 0.24).

Table 2: Comparison Of Results Of Afb Positive Cases By Two Day Strategy And One Day Method (Proportion of true positives among diseased based on older RNTCP guidelines)

Sputum Microscopy	One-Day strategy	Two-Day strategy
Positive	95 (94.05%)	101 (100%)
Negative	6 (5.95%)	0 (0%)
Total	101	101

p value = 0.01 (Chi-square test was done)

Table 3: Comparison Between One Day Strategy And Two Day Strategy - Proportion Of True Positives Among Diseased Based On Recent Rntcp Guidelines (April 2009)

Sputum Microscopy	One Day strategy	Two Days strategy
Positive	99 (98.01%)	101 (100%)
Negative	2 (1.99%)	0 (0%)
Total	101	101

p value = 0.24 (Chi-square test was done)

Based on old RNTCP guidelines, case detection rate was found to be 40.4% and 38% with gold standard and one day test method respectively (p value =0.58). Based on new RNTCP guidelines, case detection rate was found to be 40.4% and 39.6% with gold standard and one day test method respectively (p value =0.85). Both methods were found to be statistically identical in the detection of pulmonary tuberculosis (PTB) cases.

DISCUSSION

WHO estimates that TB, one of the most serious infectious diseases would be responsible for claiming millions of lives world wide in the next ten years.[1] Delay in diagnosis has a significant impact on the patient by increasing severity of the disease, drug resistance, morbidity and mortality with high risk of person-to-person transmission in the community. Medical colleges in co-ordination with RNTCP, play an essential role as an important source for TB case detection.[10] Sputum microscopy by Ziehl Neelsen staining remains the corner stone of case detection for demonstrating AFB but is associated with variable sensitivity in case detection and a high rate of patient drop-out during the diagnostic process.[4] Patient

compliance along with prompt and quality diagnosis are important aspects of RNTCP.[1] Various studies have concluded that screening TB suspects using two sputum smears is as effective as screening using three smears for diagnosis with a prerequisite of functional external quality assurance (EQA) system with blind rechecking.[11,12,13,14] Though new RNTCP guidelines recommends collection of two samples, it requires patient visit to health facility for two consecutive days for submission of spot and early morning samples. Van Deun et al found 60.4% had incomplete diagnostic series and 10% failed to return the next day with two-day strategy.[15] Similar studies in Malawi concluded that the case detection was only 48%.[16]

Sputum smear microscopy results:

Case detection rate of the present study was found to be consistent with that of Hirao et al (41%),[17] and Ozkutuk A et al (42%).[11] Studies conducted by Rohit Sarin et al (24.9%)[18] and Joshi et al (26.4%)[19] showed very low case detection rates by the routine two-day strategy which can be improved by one-day strategy. Statistical analysis in present study based on old guidelines showed a slight compromise in

sensitivity by one-day test method compared to two-day strategy (p value = 0.01). This may be due to the small sample size of the study. Based on new RNTCP guidelines, statistical analysis of our study (p value = 0.24) showed that one-day test approach was equally effective in identifying cases as the two-day method, suggesting that PTB can be efficiently diagnosed in a single day. A similar study conducted in Ethiopia by Cambanis *et al* detected 49/52 (94%) cases by the same day method and 51/52 (98%) cases by standard methods.[20] A similar study in Abuja by Hirao *et al* identified 44/78 (56.4%) and 45/78 (57.7%) of the total bacteriologically positive patients by same day and standard methods respectively.[17] The average yield of two spot specimens in two more studies was 92.2%. [5] Yassin *et al* stated that the paradoxical finding of a lower positive yield from morning sputum samples may be due to lack of supervision.[21] According to Harries *et al* and Yassin *et al*, examining two samples would reduce service and patient's cost and smears by 29.9%. [13,21]

Demonstration of AFB in sputum is the easiest, quickest and a reliable tool for diagnosis of PTB. Approach based on submission of two spot specimens on same day at an hour interval would result in significant reduction in workload of laboratories, improve quality of smear microscopy, reduce costs, improve patient compliance without significant loss of sensitivity, provide same day results and would finally increase case detection. It was concluded from the present study that submission of two specimens on the same day was equally effective as two-day method in detection of sputum smear positive TB cases.

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