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Rapid slide culture to diagnose smear negative pulmonary tuberculosis under the Revised National Tuberculosis Control Programme

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Abstract

Background and Objectives: Sputum Microscopy is the back bone for case detection under RNTCP (Revised National Tuberculosis Control Programme). However, many patients with pulmonary tuberculosis go undetected if sputum microscopy alone is done. To study the usefulness of the Rapid Slide Culture (RSC) in detecting Mycobacterium tuberculosis (M.tb) in initially sputum negative (ISN) patients under RNTCP.

Materials and Methods: During September 2006 and August 2007, out of 1560 patients who were smear negative for Acid Fast Bacilli (AFB) at the RNTCP centre of R.L. Jalappa Hospital, Kolar, 156 patients (10%) were drawn by systemic sampling method. Three sputum samples from each of these 156 ISN patients were subjected to microscopy on a concentrated sputum smear, RSC, and culture on Lowenstein-Jensen (LJ) medium.

Results: Microscopic examination of the concentrated sputum smear did not detect any positives. Sputum of 2 (1.3%) of the 156 patients tested, were found to have M.tb by both RSC and growth on LJ medium. It took an average of 34 days to detect mycobacterial growth on LJ medium, but only 7 days by RSC.

Conclusion: In the sample of patients tested, RSC was found to be as sensitive as culture on LJ medium if two sputum samples were used. It may be a useful technique to provide bacteriological evidence in smear - negative tuberculosis patients, especially when the clinical suspicion is high.

Key words: smear-negative pulmonary tuberculosis, Rapid Slide Culture, RNTCP.

Introduction

Tuberculosis is probably the most important infectious disease of human beings(1). In India about 2 million people develop active disease and half a million die every year due to tuberculosis. The situation is further compounded by the association of HIV infection with tuberculosis (2). The emphasis under the Revised National Tuberculosis Control Programme (RNTCP) is on quality sputum microscopy for early case detection and institution of Directly Observed Treatment-Short Course (DOTS). Microscopy forms the backbone of RNTCP in case detection(3,4). To improve the sensitivity of bacteriological diagnosis newer laboratory methods, including rapid molecular detection methods, have also been explored .The sophistication and especially the cost, however, make many of them beyond the reach of most of the laboratories (5,6). Rapid Slide Culture (RSC) is a safe, simple, and easy method with results available in a week's time (7,8). There have been no systematic studies using RSC on patients suspected of pulmonary tuberculosis and whose

sputum is negative for AFB by direct microscopy as screened under the RNTCP programme. Many times we require further evaluation of patients who are smear negative but have a strong suspicion of tuberculosis. In such cases, conventional culture on LJ medium takes 6-8 weeks for the primary isolation of the organisms. In this context, we undertook a study to see whether RSC, can complement direct microscopy in improving case detection.

Materials and Methods

Between September 2006 and August 2007, 1730 patients in the age group of 18-60 years, who had cough for 3 weeks or longer, were screened for the presence of AFB in their sputum as per the RNTCP guide lines(9). AFB were detected in 170 (9.83%) of the patients and 1560 (90.17%) were negative for AFB in their sputum. Of these 1560 initially sputum negative (ISN) patients, 156 (10%) were selected by the systematic random sampling method, by including every 10th patient among sputum negative patients. Children (<18years) and adults who were above 60 years were not included in the study. Care was taken to exclude patients who were on anti-tubercular treatment and those who were treated earlier for tuberculosis. Three consecutive sputum samples (1st: spot, 2nd: early morning, 3rd: spot) collected from each of the 156 patients, selected as above, were concentrated by the modified Petroff's method(9). The total number of samples, thus concentrated, numbered 468.The concentrated deposits of sputum obtained were divided in to 3 parts. The smear made from the first part was stained by the Ziehl-Neelsen's (ZN) method(9) and examined for AFB. The second part was subjected to RSC for AFB and the third part was cultured on 2 bottles of LJ medium. For the purpose of RSC(7), the sputum concentrate was smeared on the lower one third of a sterilized glass slide; air dried, dipped in 10 ml of human blood medium, and incubated for 7 days at 37°C. The human blood medium was prepared by using outdated (but stored for not longer than 4 weeks) citrated blood obtained from the Blood bank. The blood was diluted with equal volume of sterile de-ionized water to cause haemolysis. To the haemolysed blood, antibacterial and antifungal agents (Polymyxin B: 200,000units/L, Carbenicillin: 100mg/L, Trimethoprim: 10mg/L and Amphotericin B: 100mg/L) were added to make the medium selective. The pH of the medium was adjusted to lie between 6.5 and 7.5 and it was dispensed in 10 ml quantities in sterile screw capped wide mouthed reagent bottles. On the 7th day, the slide incubated in the human blood medium was taken out, washed in distilled water, and dried in an oven at 80°C for 30 minutes. It was stained by the Z-N method and was examined under the oil-immersion objective. The growth, if any, was graded as 1+, 2+, 3+, &4+ according to Purohit et al(10). As a positive control, H37Rv strain of M.tb was processed with each batch of specimens. If any sputum was found positive, it was re-tested, using a sputum aliquot stored at +4°C; this confirmed the reliability of the technique. The two bottles of LJ medium inoculated and incubated at 37°C were inspected for growth at the end of every week. The growth, if any, at the end of 12 weeks was graded as 1+, 2+, or 3+. The colonies on LJ medium were confirmed as M.tb by the Niacin test (Niacin test kit, HI media) Growth on LJ medium was considered as Gold standard to calculate the sensitivity and specificity of RSC.

Results

Out of 156 patients studied 106 (67.9%) were males and 50(32.4%) were females. The majority of patients were in the productive age group of 20-50 years (Table 1). Microscopic examination of the stained concentrate of the sputum, did not detect any more positives. The RSC and culture on LJ medium, both, detected 2 more sputum positive patients among the 156 (ISN) patients tested, who were missed by the direct smear examination. The bacterial colonies on LJ medium were confirmed as M. tb by the Niacin test(11). Details of results obtained on sputum culture of the 2 patients found positive by both RSC and LJ medium are presented in table 2. Thus when two samples were

Table 1. Age and gender distribution of patients

Age	Male (%)	Female (%)
21-30	24(15.4%)	08(05.1%)
31-40	38(24.4%)	14(09%)
41-50	30(19.2%)	20(12.8%)
51-60	14(09%)	08(05.1%)

Table 2: The results of sputum culture in RSC and on LJ medium of the 2 patients detected to be positive.

Patient	Sample	Grading of growth on RSC*(day when growth was seen)	Grading**of growth on LJ medium (day when growth was seen)
Patient 1	1	3+(7)	2+(36)
	2	4+(7)	3+(30)
	3	3+(7)	3+(30)
Patient 2	1	3+(7)	2+(30)
	2	3+(7)	2+(36)
	3	Negative	1+(42)

*Growth in RSC was graded according to Purohit et al (10)
** Growth on LJ medium was graded according to RNTCP (9)

tested (one spot and one early morning), RSC showed sensitivity and specificity comparable to culture on LJ medium. The time taken for growth of AFB varied widely between RSC and culture on LJ medium. RSC showed growth in 7 days, where as LJ medium took an average of 34 days. Clinical records of the 2 sputum- negative patients, who turned out to be culture positives, were reviewed. One of the patients had cough, fever, and radiological changes suggestive of tuberculosis and another had cough and weight loss with evening rise of temperature for 20 days without any radiological changes in chest X-ray. Both these patients did not have infection with HIV as evidenced by serology.

Discussion

RSC, though described in the 19th century by Robert Koch, could not be practiced because of the problems of bacterial and fungal contamination of the media. In the recent years these problems have been overcome with the incorporation of antibiotics and antifungal agents in the media(7). Mycobacterium grows faster in liquid medium and the growth is identified by the characteristic cord formation. Non tuberculous mycobacteria do not give a positive RSC as non tuberculous mycobacteria lack the ability of cord formation with the exception of *Mycobacterium chelonae(12)*. RSC, as borne out by our study and those done by others, is more sensitive compared to direct smear microscopy(7,8). However the AFB detection rate

of 1.3% among ISN patients in our study is low compared to earlier studies which detected 8.9%-11% of ISN patients to be positive by RSC(7,8). These studies had included only ISN patients who had shown x-ray changes suggestive of pulmonary tuberculosis. We had not made any such patient choice in our study. Thus the disparity in the results may be due to the difference in the selection criteria. The earlier studies did not conform to the RNTCP guidelines and thus positives missed in direct sputum microscopy of a single sample might have been picked up by RSC, which gave a higher detection rate. Among the sputum negative patients who were positive RSC, we found that, one of them had X ray changes suggestive of tuberculosis and another had loss of appetite and evening rise of temperature, clinically strongly suggestive of tuberculosis. We think that RSC can be useful adjunct in diagnosing tuberculosis in such patients who remain sputum negative by direct microscopy. To conclude RSC was found to be as sensitive as culture on LJ medium for the diagnosis of smear-negative tuberculosis when 2 sputum samples were used. In a referral Hospital setting, as in this study, RSC detected 1.3% of ISN patients to have pulmonary-tuberculosis. The turn around time was seven days. The test needs to be evaluated in a larger sample of smear-negative patients, with or without radiological findings clinically suggestive of pulmonary-tuberculosis, who remain clinically ill but sputum - smear negative; RSC could provide bacteriological evidence of pulmonary tuberculosis in such patients.

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