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Original Research Article

Laboratory Evaluation of a New Rapid Slide Culture (RSC) Technique for Diagnosis of Extra-Pulmonary Tuberculosis

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ABSTRACT

Introduction: Tuberculosis is an infectious disease caused mostly by *M. tuberculosis*. The bacterium mostly affects lungs but it can affect other parts of the body also. Diagnosis of pulmonary TB is easier than diagnosis of extra-pulmonary TB (EPTB) because of large number of large number of mycobacterium present in sputum sample. Diagnosis of EPTB is difficult depending on liquid culture *i.e.* MGIT and molecular methods. These methods are time consuming and costly so we have tried a new slide culture method which is cost effective and less time taking.
Materials and Methods: Total 100 patients of suspected EPTB were included in this study after written consent. Menstrual blood, lymph node biopsy and urine samples were subjected for the study of cytology, smear microscopy, MGIT and four sets of RSC.
Result: Out of 100, 24 lymph node biopsy and 13 genital samples were found positive for granuloma cells. Direct smear microscopy has showed 5 AFB positive. Total 11 samples were found culture positive by MGIT culture method. RSC has showed 4 positive cases. Sensitivity and specificity of RSC were recorded 36.4% and 100% respectively. Positive Predictive value and negative predictive value were 100% and 92.5% respectively.
Conclusion: Earlier RSC was used for detection of Mycobacterium growth in only sputum samples. After some improvement we used it for diagnosis of EPTB. In present study the sensitivity of RSC was to 36.4%, which is not acceptable for diagnosis of EPTB but positive predictive value (100%) leaving a hope to improve the RSC technique for diagnosis of EPTB.

Key words: Rapid Slide Culture, RSC, extra-pulmonary TB, EPTB, Tuberculosis.

INTRODUCTION

Tuberculosis is an infectious disease caused by *Mycobacterium tuberculosis*. The bacterium mostly affects the lungs, leading to pulmonary tuberculosis (PTB) but it can affect other parts of the body known as extra-pulmonary TB (EPTB). EPTB refers to TB involving organs other than the lungs for example pleura, genitourinary tract, lymph nodes, abdomen, bones, CNS etc. A patient with both pulmonary and EPTB is classified as a case of PTB For example, miliary TB is classified as PTB because there are lesions in the lungs. On the other hand, tubercular intrathoracic lymphadenitis or tuberculous pleural effusion, without radiographic abnormalities in the lungs, constitutes a case of EPTB. [1,2]

According to the Revised National Tuberculosis Control Program (RNTCP)

report 2018, there were 27, 90,000 reported cases of TB (211 cases per 100,000 people) in India. Approximately 20 percent of these are EPTB. The most commonly affected sites of EPTB in India were pleura, followed by lymph nodes, gastrointestinal organs, bones and joints, central nervous system (CNS), and genitourinary organs. [3]

Cultivation of Mycobacterium tuberculosis from a specimen obtained from the patient is a gold standard diagnostic method. Diagnosing EPTB is difficult because clinical samples obtained from relatively inaccessible sites may be paucibacillary, decreasing the sensitivity of diagnostic tests. Since the direct smear microscopy has a low sensitivity with a range of 0% to 40%, negative results cannot exclude the presence of Acid Fast Bacilli. The sensitivity of Mycobacteria culture vary from 30% up to 80%, but it usually takes 2 to 8 weeks to obtain the result, which is too slow to take a decisions of treatment. [4]

Diagnosis of EPTB poses a challenge to the clinician due to its variety of presentations and insidious onset which does not bring the patient to the physician at an early stage of disease. Absence of typical clinical features and often negative conventional diagnostic tests (smear microscopy and culture) due to paucibacillary nature of samples also contribute to delay in diagnosis. [5]

Molecular diagnostic methods are rapid and sensitive but expensive. Thus a new cheap, rapid and sensitive diagnostic method is required for EPTB. Rapid slide culture (RSC) could be fulfilling this requirement. So, in this study we focused on double culture method including Liquid culture and RSC technique for early diagnosis of tuberculosis.

MATERIALS AND METHODS

The present study was a prospective study conducted in the Departments of Microbiology, Rama Medical College, Hospital and Research Centre over a period of years (2015-2017) after institutional ethical clearance.

Sample size

A total of 100 patients were enrolled in our study after written informed consent. They were 30 cases of suspected renal tuberculosis. 30 suspected tubercular meningitis patients. 20 suspected tubercular lymphadenitis (TBL) cases and 20 suspected genital tuberculosis cases were also included in the study.

Inclusion criteria: All clinically suspected cases renal TB, tubercular lymphangitis and genital tuberculosis.

Exclusion criteria:

1) Patients below age group of 14 years.
2) Sample less than 0.5 ml of sample.

The samples were subjected to (i) Smear microscopy by Zeihl-Neelsen stain (ii) Cytology for granulomatous cell (iii) Culture for Mycobacteria by RSC method and (iv) culture for Mycobacteria by MGIT method. Samples were processed by Universal Sample Processing (USP) method in tuberculosis laboratory. [6]

All samples of fine needle aspirates from lymph node and first and second day menstrual blood were collected using aseptic precautions in sterile falcon tube containing sterile Middle brook 7H9 broth with malachite green and OADC growth supplements. The falcon tubes were incubated at 37^0C for two days.

In case of renal tuberculosis three days of early morning clean catch urine was collected in falcon tubes. The urine samples were centrifuged at 3000 rpm for 20 minutes and deposit were inoculated same as the above. The tubes were incubated at 37^0C for two days.

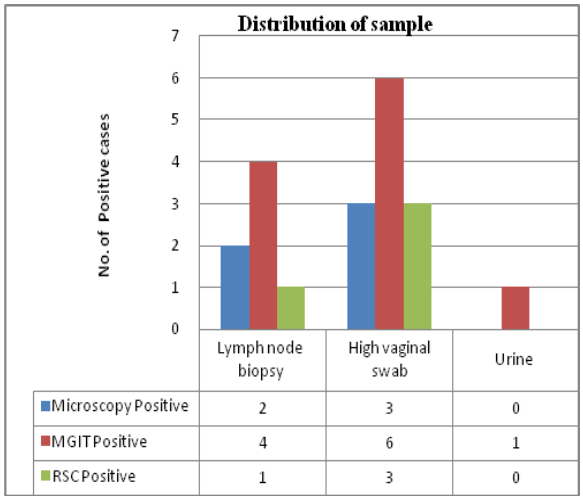
After two days of incubation samples in the falcon tubes were digested and decontaminated by NALC NaOH method followed by centrifugation at 3000 g for 15 minutes. [6] Smears were prepared aseptically on sterile longitudinally split glass slides at lower one third of the slide in biosafety cabinet IIb. The smears were aired dried and inserted in Makarthy tube containing sterile Middle brook 7H9 broth with PANTA antibiotics and OADC growth

supplements (Himedia). Such four sets were prepared for each sample and observed after Z-N stain for acid fast bacilli on 3rd, 6th, 9th and 12th day of incubation.

Centrifuge deposit was also culture by Mycobacterium growth indicator tube (MGIT) method. The MGIT culture was observe daily by manually operated machine daily till 42 days. In case of positive culture, Z-N stain was performed for AFB. Once AFB is detected the MGIT tube is subculture in another MGIT tube containing para-nitro benzoic acid for differentiation of Non tubercular mycobacterium form MTB.

RESULT

The distributions of the 100 extra-pulmonary samples were processed as follows: 62 samples were collected from cervical lymph nodes, similarly 30 or menstrual blood samples and 8 urine samples. Out of 62 lymph nodes only 24 cases (38.7%) were found positive for granulomatous cells and direct AFB was detected in 2 cases (3.2%). 4 (6.4%) samples of lymph node biopsy were found positive MGIT culture. The time taken by MGIT culture method ranges from 14 days to 21 days. Only one case (1.6%) of Lymph node biopsy was found positive for *Mycobacterium tuberculosis* by RSC culture method.



Out of 30 suspected genital tuberculosis cases in 13 (43.3%) cases granulomatus cells was detected. Direct AFB was detected in 4 (13.3%) cases of suspected genital tuberculosis. Mycobacterium growth was detected in 6 (20%) and 3 (10%) by MGIT and RSC methods respectively. The time taken by MGIT and RSC culture methods was 14 days (ranges 7-21 days) and 12 days respectively.

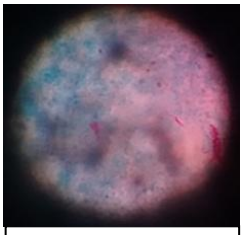
No AFB was detected in 8 cases of suspected renal tuberculosis cases. Mycobacterium growth was detected in only one (12.5%) case of suspected renal tuberculosis, where all cases were negative for mycobacterium growth by RSC method.

Table No. 1

Test		MGIT culture positive	
		Positive	Negative
RSC	Positive	4	00
	Negative	7	86
TOTAL		11	86

Sensitivity and specificity of RSC in comparison to MGIT were calculated as 36.4% and 100% respectively.
Positive Predictive value and negative predictive value were calculated as 100% and 92.5% respectively.

RSC culture



Growth in RSC

DISCUSSION

Diagnosis of EPTB is challenging in a clinical laboratory. The conventional methods like direct AFB smear and culture are not always positive because of few numbers of bacilli in the extra-pulmonary samples like lymph node biopsy, CSF, Pleural fluid etc. *Mycobacterium* culture by MGIT is also come positive rarely and require more time, in present study average time 14 days (ranges from 7 to 21 day). Hence in our study we tried to reduce the time of *Mycobacterium* growth detection by

using RSC technique. RSC was first introduced by Robert Koch for MTB growth detection, he succeed to grow *Mycobacteria* in seven days. [7] Further RSC was improved by Dickinson and Mitchison. [8] Gupta *et al.*, substituted fluorescence microscope with bright field microscope. [9] RSC was used for detection of Mycobacterium growth in only sputum samples. After some improvement we used it for diagnosis of EPTB.

In present study 100 cases of clinically suspected extra pulmonary tuberculosis were enrolled after informed written consent and who were not on ATT. The patients were followed up at least till the two months of intensive phase of therapy to see its response of therapy. The patients were of above 14 year of age groups and both sexes were included in this study. Common signs and symptoms like low grade fever, anorexia, malaise is seen in most of cases that improved with therapy.

Direct smear stained by Ziehl-Neelsen stain for AFB was positive in 31.2% EPTB samples. It has been reported that a positive smear requires about 10^4 bacilli/ml of sample without concentration. [10]

Mycobacterial growth was positive in 11% cases of EPTB by MGIT culture method; it may be due to paucibacillary nature of extra-pulmonary sample. Singh *et al.*, [11] has reported 14% positive culture. A study by Sinha *et al.*, [12] has found culture positivity in 20% of cases. In present study the RSC technique gave only 4% positive growth it may be due to small amount of sample and four sets of RSC. Though the time taken by RSC was 12 days, which was less than the MGIT culture method more over cost of the test of RSC is less than the MGIT which was Rs. 50 on an average.

CONCLUSION

In present study the sensitivity of RSC is found to 36.4%, which is not acceptable for diagnosis of EPTB but has specificity to 100%. Positive predictive value was found 100% and negative predictive value was recorded 92.5% leaving a hope to improve the RSC technique for diagnosis of EPTB. In future RSC may be an option for diagnosis of EPTB as it is less time taking and cheaper process than other culture methods.

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REFERENCES

1. World Health Organization. Definitions and reporting framework for tuberculosis: 2013 revision (updated December 2014). Geneva: World Health Organization; 2013.
2. Ji Yeon Lee “Diagnosis and Treatment of Extra-pulmonary Tuberculosis” Review article, Tuberc Respir Dis 2015; 78: 47-55.
3. TB India report 2018.
4. Canadian Thoracic Society and the Public Health Agency of Canada and Licensors. Canadian tuberculosis standards. 7th ed. Ottawa: Public Health Agency of Canada; 2013.
5. Anju Jain “Extra Pulmonary Tuberculosis: A Diagnostic Dilemma” Ind J. Clin Biochem 2011; 26(3): 269-273.
6. Betty A. Forbes, Daniel F. Sahm, Alice S. Weissfeld. Bailey & Scott’s Diagnostic Microbiology. Mycobacteria. USA: Mosby; 2002. p. 538-71.
7. Koch, R, Uber die Aeliologic der Tuberkulose, Bed. Kin. Wschnschr in Berry JW and Lowry Hope DH: A slide culture method for early detection of growth of the tubercle bacillus. Am. Rev. Tuberc. 1949, 60, 51.
8. Dickinson J.M. and Mitchison D.A: New slide culture sensitivity tests. Proceedings of the XXV World Conference of IUAT, Buenos Aires. 1982; 15 (18): 180.

9. Gupta P.R., Singhal B, Sharma T.N. and Gupta RB: A modified slide culture technique for quick drug sensitivity testing of M. tuberculosis. Ind. J. Tub. 1992; 39: 113.
10. Kant L. Improving detection of infectious cases. Indian J Tuberc. 2001; 48:115-6.
11. Singh KK, Muralidhar M, Kumar A, Chattopadhya TK, Kapila K, Singh MK, et al. Comparison of in house polymerase chain reaction with conventional techniques for the detection of Mycobacterium tuberculosis DNA in granulomatous lymphadenopathy. J Clin Pathol. 2000; 53:355-61.
12. Sinha SK, Chatterjee M, Bhattacharya S, Pathak SK, Mitra RB, Karak K, Mukherjee M. Diagnostic evaluation of extrapulmonary tuberculosis by fine needle aspiration (FNA) supplemented with AFB smear and culture. J Indian Med Assoc. 2003; 101:588-91.

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