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EVALUATION OF A NEW RAPID SLIDE CULTURE (RSC) TECHNIQUE FOR EARLY DIAGNOSIS OF PULMONARY TUBERCULOSIS

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ABSTRACT

Introduction: Pulmonary tuberculosis (PTB) is one of major cause of mortality among the infected population. Ziehl- Neelsen staining method is a commonly used low cost, rapid test but it has low sensitivity and specificity. Rapid slide culture (RSC) could be a low cost alternative culture method for rapid diagnosis of PTB.

Materials and Methods: This was a blinded prospective diagnostic trial. A total of 297 patients with suspected active PTB and direct AFB smear negative were enrolled in this study. Direct smear and smear after concentration were examined for AFB. Sample were inoculated in one MGIT system and four RSC.

Results: Out of 288 cases, 63 (21.9%) were diagnosed as PTB that were culture positive for M. tuberculosis due to growth in MGIT culture system. RSC was Positive in 54 (18.8%) cases that were culture positive for M. tuberculosis.

Discussion: In present study the time required for RSC was 6-12 days. None of the other studies Middlebrook 7H9 and malachite green was used, which makes RSC more sensitive and specific for Mycobacterium tuberculosis growth.

KEYWORDS :

INTRODUCTION

Pulmonary tuberculosis is one of major cause of mortality among the infected population. Approximately one third population of the world is infected with the *Mycobacterium tuberculosis*, a causative agent of tuberculosis[1]. India is the most TB prevalent country in the world, probably because of delay in diagnosis of the pulmonary TB cases which is the main sources infection. It is believed that one open case of pulmonary TB infect ten persons in his life time if not treated timely. Early detection of *M. tuberculosis* from clinical sample is required for treatment at an early stage of the disease. [2,3]

Direct microscopic examination for detection of acid fast bacilli (AFB) is a common method for TB diagnosis. Two staining methods, Ziehl- Neelsen and Auramine-Rhodamine are used for demonstration of AFB. Ziehl- Neelsen staining method is a commonly used low cost, rapid test but it has low sensitivity and specificity. [4, 5, 6]Though Auramine-Rhodamine florescent staining method increases the sensitivity but require an expensive LED microscope. Concentration of the sputum increases sensitivity of the microscopic detection of AFB but again increases the cost of test and labor. Also Z-N staining method cannot differentiate *M. tuberculosis* from Non-tuberculosis Mycobacterium (NTM). [7]

A definitive diagnosis of TB is therefore dependent on culture followed by a battery of biochemical tests. Conventional culture methods utilize solid media Lowenstein-Jensen and liquid media e.g Middlebrook 7H9 broth. Though culture method is gold standard but it require 2- 8 weeks as *M. tuberculosis* is a slow growing bacteria and additionally one week is required for biochemical tests. This delay in diagnosis may increase the chances of spread of infection. Automated culture system e.g MGIT, BACTEC and molecular techniques GeneXpert are rapid and sensitive but are costly therefore microbiologist are looking for a low cost, rapid and sensitive TB diagnostic technique such as Rapid slide culture (RSC) method. [4, 8]. Robert Koch, who has discovered the causitive agent of TB, introduced RSC technique. He successed to grow *M. tuberculosis* in seven days but high rate of contamination hindered further success. Dickinson and Mitchison used RSC for drug susceptibility of *Mycobacterium tuberculosis*. They employed fluroscent microscope. [9] P.R. Gupta et al. used fluroscent microscope instead of bright field microscope. Though RSC is sensitive, evaluated in several studies but human blood medium used is neither ethical nor available as much as required.

In the present study, we employed Middlebrook 7H9 broth instead of human blood medium as RSC culture medium and modified the growth detection technique. We evaluated whether pulmonary TB can be diagnosed using this new RSC technique.

AIMS AND OBJECTIVES

- Comparison of Rapid Slide Culture (RSC) method with MGIT culture method.
- To find out the sensitivity and specificity of RSC method in comparison with the MGIT culture method.

MATERIAL AND METHODS

This study was conducted at Rama Medical College, Hospital & Research Centre, Kanpur (U.P.) India from June 2015 to November 2016. This was blinded prospective diagnostic trial. A written patient informed consent was obtained from all recruited patients before sample collection. Total 297 patients showing minimum clinical and radiological evidence of pulmonary tuberculosis were selected for the study. Patient below 15 years of age, unable to produce minimum 5 ml of sputum, known HIV cases and received anti-TB therapy were excluded from the study.

Patients were asked to collect sputum sample in a wide mouth container. All the samples were screened by Ziehl-Neelsen staining method afterward desired patients were selected for the study. Minimum 5 ml of sputum was collected in Falcon tube containing 1 ml of sterile Middlebrook 7H9 broth with OADC growth supplement. All Samples were stored at -20 C till the processing.

Samples were processed in bio-safety cabinet level 3. Once in week samples were decontaminated and concentrated by NALC-NaOH method. One smear was prepared from each sample on a glass slide, heat fixed and stained by Ziehl-Neelsen staining method. The slide was examined and results were recorded as AFB (+ve/–ve) after concentration. Each sample was inoculated in MGIT culture system and four sets of RSC. In case of culture positive subculture was done in MGIT containing Para Nitro Benzoic acid to differentiate Mycobacterium tuberculosis from Non-tuberculosis Mycobacterium (NTM).

Cuture Media for the RSC technique, containing MiddileBrook 7H9 broth (HiMedia)**,** OADC growth suppliment (HiMedia) **and** PANTA antibiotics (Bicton&Dikson). 10 ml of RSC media (9 ml of Middlebrook and 1ml OADC supplement and 0.1 ml of 0.04 malachite green%) was kept in a Makarthy tube. RSC media was prepared before one day of sample processing and stored at 8 ˚C.

Rapid Slide cultur Technique

Glass slides which were split longitudinally, marked sample number with glass slide, placed in glass petry plate and sterilized by hot air oven at 160 ˚C for 30 minutes. One smear (1 cm x 2cm) was made on lower 1/3rd of a slide. The smears were aired dried and then placed in a Makarthy tube containing RSC media. The Makarthy tube was placed in a plastic box in a slant position at an angel of 30˚. The slides were pleased in such a manner that smears facing upward. For one sample four RSC were prepared. All tubes were incubated at 35 ± 2 ˚C. RSCs were observed on 3rd, 6th, 9th, and 12th day respectively. After incubation period slides were taken out with the help of forceps afterward placed them in hot air oven at 50 ˚C for 10 minutes. Slide were treated with ethanol and stained by Ziehl-Neelsen staining method. The whole smear was scanned under 10 X objective lens for any red spot. Once red spot was observed, then focused it under 100 X (oil immersion) objective lense for morphological confirmation of the acid fast bacillus. An in house Mycobacterium tuberculosis culture stain was cultured by RSC in each batch of RSC media as positive control.

The growth detection time was recorded for both cultures. Statistical analysis and rate of contamination was calculated using McNemar test (OR CI 95% P<0.001) and comparative time to detect mycobacterium growth by t test (P<0.001).

RESULT

A total of 297 patients with suspected active pulmonary tuberculosis (PTB) and direct AFB smear negative were enrolled in this study. After exclusion of 7 cases during the study due to contamination and 2 NTM isolates, 288 cases were included in the final statistical analysis. Of these 288 cases, 63 (21.9%) were diagnosed as PTB that were culture positive for M. tuberculosis due to growth in MGIT culture system. Out of 288 included cases, 27 (9.4%) were shown AFB positive after sputum concentration. RSC was Positive in 54 (18.8%) cases that were culture positive for M. tuberculosis (Table 1)

Table 1. Distribution of positive results according to test used.

Test	Positive	Percentage
Smear positive after concentration	27	9.4%
MGIT	63	21.9%
RSC	54	18.8%
All the 3 tests	19	6.6%
One or more tests	69	23.9%

When compared the growth of two culture techniques, MGIT and RSC the results are as follows: Total 51 (17.7%) cases were found to be culture positive and 224 (77.7%) culture negative by both the culture techniques, where as 63 (21.9%) MGIT and 52 (18.0%) RSC were found to be positive separately (Table 2). The sensitivity and specificity of RSC in comparison with MGIT culture technique were 80.9 % and 99.6 % respectively.

Table 2. Comparison of Rapid slide culture technique with MGIT growth

RSC growth	MGIT culture		
	Positive	Negative	Total
Positive	51	1	52
Negative	12	224	236
Total	63 (21.9%)	225 (78.1%)	288 (100%)

The comparison of AFB smear after concentration with the MGIT culture technique showed a sensitivity of 34 % and specificity 96.9% (Table 3).

Table 3. Comparison of AFB smears after concentration with MGIT

AFB after conc.	MGIT culture		
	Positive	Negative	Total
Positive	22	7	29
Negative	41	218	258
Total	63 (21.9%)	225 (78.1%)	288 (100%)

Out of 288 samples, 29 samples showed AFB positive after concentration. In comparison of MGIT, RSC culture techniques showed 78.0% sensitivity in smear negative cases (Table 4.)

Table 4. Correlation between RSC and MGIT culture result in smear negative after concentration cases

RSC growth	MGIT culture		
	Positive	Negative	Total
Positive	32	1	33
Negative	9	0	9
Total	41 (97.6%)	1 (2.4%)	42 (100%)

MGIT and RSC culture techniques take maximum time 42 days and 12 days respectively. In this study MGIT took maximum 28 days and maximum samples growth appear at 14th day, where maximum numbers of sample showed growth by RSC culture technique in 6th day.

Discussion

Diagnosis of active pulmonary tuberculosis at an early stage of disease has aim of the Revised National Tuberculosis Control Programmed (RNTCP) in India to prevent the spread the disease. Tuberculosis is considered as a disease of low socioeconomic population, where rapid and low cost test to diagnosis PTB will be helpful for the TB control Programme. Direct smear examination by Z-N stain has been the backbone of any TB control programme. Diagnosis of PTB by Z-N stain is rapid but less sensitive method. At an early stage of active PTB direct smear examination often comes negative, however concentration and fluorescent microscopy increases the sensitivity but these are less sensitive than gold standard culture method (LJ and MGIT). Conventional culture method either more costly like MGIT or takes too much time like LJ media. This may lead to delay in treatment and promoting the spread of the disease in the society.

The Rapid Slide culture technique was invented by Robert Koch, could not be practiced because of high rate of bacterial and fungal contamination. In last few years, this problem was solved up to some extent by using antibacterial and antifungal agents those are not inhibitory for Myco-bacterial growth. In most of such studies shown that RSC is capable of detecting M. tuberculosis in 9-11 % smear negative cases.

The present study was focused on direct smear negative suspected active pulmonary tuberculosis, where as in similar other studies both direct smear negative and positive cases have been included this might be the region that in present study RSC gained less positive result as compared with other studies. Hemvathi et al and

Sanjeev H. et al. faced some problem like washing of the smear in RSC medium, so that keeping this problem in our mind we avoid shaking of the Mac Karthy tube and kept in slanting position to prevent the washing of the smear. In this study few smear positive cases showed RSC negative may be because of over drying.

In previous studies old citrated human blood media (HBM) with PANTA antibiotics were used. Human blood is neither easly available nor chemically defines. PANTA antibiotic is costly which may increase the cost of test. Middle brook 7H9 medium used in this study is a transparent medium and malachite green used to prevent contamination is very cheap. In previous Mac Cartney bottle were used of which mouth is narrow than the tube used in this study Makarthy tube, that provide a wide area of slide to observe the growth of slide.

RSC technique has a drawback that once the growth is observed can't re-incubate. In most of the previous studies a fixed seven days of incubation was there in RSC except Ramesh alias Thirumalesh D H (2014), who has taken two sets of RSC, one was observed on 7th day and another was on 13th day. This additional step might increase the sensitivity of RSC (100%) in comparison with LJ media which is more than the RSC (65.2%) reported by Jena and Rajan in 1995.

In present study the time required for RSC was 6-12 days, 14-21 days for growth in MGIT system. None of the other studies Middilebrook 7H9 and malachite green was used, which makes RSC more sensitive and specific for Mycobacterium tuberculosis growth. Some NTM growth was observed in this study, by adding PNB in an additional set we can make RSC more sensitive for M. tuberculosis complex, where M. tuberculosis can be differentiated from NTM.

The cost of the PTB diagnosis by MGIT method was found to be much more expensive (RS. 700) than RSC methods (Rs.50). The cost difference was also reported by other studies like Ramesh alias.

Conclusion

In present study it has been concluded that sensitivity of RSC closure to sensitive as MGIT. But when the time consumption and cost are compared, RSC is found to be much more effective than MGIT. By some modification we can make RSC as sensitive as MGIT which would be easy to perform, rapid and cheap.

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