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Rapid Slide Culture Technique for *Mycobacterium Tuberculosis* and Predictors of Poor Outcomes among Pulmonary Tuberculosis Patients

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Abstract

Tuberculosis (TB), a dreadful disease known to mankind continues to be a problem in a developing country like India. The incidence of people getting infected with TB is on the rise due to compounding factors like coinfection with the human immunodefiency virus and multidrug-resistant strains. There is a definitive need for early diagnosis and treatment of TB to curb transmission of the infection. Direct smear microscopy, though cheap and rapid, lacks sensitivity. Isolation of *Mycobacterium tuberculosis* in culture requires a long time, because of which there is a need for a rapid method which has good sensitivity and specificity for the detection of *M. tuberculosis.* Hence the present study was undertaken to determine Performance of rapid slide culture method for confirming pulmonary tuberculosis in clinically suspected and smear positive cases and also to assess the factors responsible for poor outcomes in the study population

Key Words: Tuberculosis, Early diagnosis, Rapid slide culture method, Poor outcomes.

Introduction

In 2020 and 2021, the coronavirus pandemic had huge medical, social, and economic implications. This includes the number of people diagnosed with tuberculosis (TB) and reported as TB cases, as well as the availability of and access to necessary TB services.1 Years of progress in eradicating tuberculosis have been stalled by the COVID-19 epidemic, and TB fatalities have risen for the first time in over a decade. More individuals died from tuberculosis in 2020 than in 2019, with fewer people being identified, treated, or given TB preventative therapy, and total spending on key TB treatments declining. Despite the fact that tuberculosis (TB) is a preventable disease, it is one of the top 10 causes of mortality worldwide.2,3

Bacteriological examination of the clinical specimens plays an important role in the diagnosis of any mycobacterial infection, more so for TB. The widely used acid-fast smear (Ziehl Neelsen stain) for the demonstration of the acid-fast bacilli, though rapid

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and simple to perform, has a low sensitivity, especially for single and paucibacillary specimens. Also, it cannot differentiate live bacilli from inactive or dead bacilli. A definitive diagnosis of active Mycobacterial infection therefore depends on the isolation and identification of mycobacteria from the clinical specimen, by culture. Traditional or conventional methods for mycobacterial culture utilize media containing egg or potato base (Middlebrook 7H10 or 7H11) or albumin (Lowenstein - Jensen medium, LJ). Although these media support the growth of mycobacteria, several weeks (2-8 weeks) of incubation maybe necessary before the growth can be detected. This duration may further be prolonged in the case of paucibacillary specimens. Robert Koch was the first to employ coagulated human serum in a rapid slide culture (RSCJ technique). The 'gold standard' for TB laboratory diagnosis is mycobacterial culture. Lowenstein-Jensen medium (LJ) is an egg-based solid media that is cheap yet takes weeks to culture.[5] Liquid media culture, such as Middlebrook 7H12 in MGIT tubes, is quicker but costlier. The rapid slide culture (RSC) technique is an ancient technology that is gaining popularity again due to its low cost and speed.[4,6] Dickinson and Mitchison described a new slide culture technique that was rapid, simple, and safe but required the use of a fluorescent microscope. We have modified the above technique to obtain similar results with the use of a bright field microscope[16]. Rapid slide culture is a sensitive, economical, and rapid method for diagnosis. This method uses human blood medium and an incubation period of seven days. The method was successfully followed by *Jena et al.* Considering this background, we modified the growth detection approach and utilised Middlebrook 7H9 broth instead of human blood medium as the RSC culture medium in this work. We wanted to see if this novel RSC approach might be used to identify pulmonary tuberculosis quickly when compared to conventional LJ medium culture.[6,7].

Materials and Methodology

The study was carried out on a total of 40 AFB positive sputum samples collected from patients diagnosed with pulmonary tuberculosis. The samples received in Laboratory of Microbiology, JSS Hospital, Mysore. Among the 40 positive samples, the majority of them (77.5%) were male and 22.5% were female. Also, the collected samples were divided into three groups according to their age. Group I were people of the age 21- 40, group II included people between 41-60 years and above 60 years was considered as group III. Among the 40 samples, 20 belongs to group I, 14 of them belonged to group II and 6 of them belonged to group III. The majority of the cases were from the first group which accounted for about 50% of the total cases evaluated. There were no samples from less than 20 years age group for the study.

For the screening of samples did the ZN staining (Gabblet's method) and gave the grading according to RNTCP guidelines for grading ZN-stained sputum smears. After Screening The samples were decontaminated by using NALC method. 100 ml of RSC media (90 ml of Middlebrook and 10ml OADC growth supplement and 1 ml of 0.04% malachite green) was taken in a beaker and kept for autoclave sterilisation at 121˚C for 30 minutes. The media was incubated overnight for determining the contamination. If the media turns out to be contaminated after incubation, they were discarded immediately. If there was no evidence of contamination, the media was then poured into different coplin jars. Coplin jars were sterilised in hot air oven at 160˚C for 2 hours. 30 ml of the media was poured into one coplin jar and sealed with parafilm. RSC media was prepared before one day of sample processing and stored at 8 ˚C. For one sample, four RSC were prepared. The slides were first sterilised placing in a petri dish plate by using hot air oven at 160˚C for 2 hours. The sediment collected after centrifugation was taken for smear preparation. One smear (1cmx2cm) was made on lower 1/3rd of the slide. Smears were prepared for each sample without heat fixing. Then the smears were put into coplin jars containing RSC media. Only one slide was kept in one jar in a slanting position. The coplin jars were sealed with parafilm and put for incubation at 37˚C. Coplin jars were observed after 3,6,9,12days respectively. On the particular day, the turbidity of RSC media was noted. The slides were taken out with the help of forceps and heat fixed. The media left out in the coplin jar was then decontaminated using autoclave. After heat fixation, ZN staining was performed. It was then observed under light microscope by oil immersion lens for detecting the growth of MTB. To obtain pertinent data, a pre-designed data sheet was

employed. The data collected was used to investigate probable predictors of poor outcomes in pulmonary TB patients.

Results

Rapid slide culture technique for mycobacterium tuberculosis

72.5% samples showed no increase in growth at the end of 3rd day, At the end of 6th day, 1+ grading was observed in a smaller number of samples compared to 3rd day. 22.5% samples reported 1+, maximum grading was given to 2+ and 3+ which was about 30% and 35% of cases respectively. At the end of 9th day, 1+ was observed in comparatively less samples which was 17.5% of the total cases. 42.5% samples showed 2+ grading and 17.5 % samples showed 4+ grading. The least observed grading was 1+ followed by 3+ which accounted for about 22.5% of the total samples evaluated. At the end of 12th day, 1+ grading was observed the least. It was seen in only 10% cases. The maximum samples reported for a 4+ grading compared to other days. 7.5% samples reported 4+, 30% reported 3+ and 32.5% reported 2+.

The number of samples that reported 4+ grading kept on increasing when evaluated after incubation on consecutive days. Maximum number of samples reported 4+ grading after the 12th day. To conclude, 62.5% samples showed growth of different grading at the end of 12th day. Whereas, 37.5% samples no growth even at the end of 12th day. (Table 1)

Table 1: Showing RSC growth at the end of 12th day

RSC growth	n	%
Positive	25	62.5
Negative	15	37.5

The number of bacilli in 20 OIF was observed at the end of 3rd, 6th, 9th and 12th days. The number showed an increase in each of the respective days. The maximum number of bacilli was thus noted at the end of 12th day (Table II).

Table 2 showing Increase in number of bacilli

	Mean number of bacilli	SD
3rd day	54.5	12.02
6th day	127.5	10.6
9th day	276	90.5
12th day	430	98.99

Predictors of poor outcomes among pulmonary tuberculosis patients

Among 40 patients, majority reported cough as the primary symptom for them to visit a health centre. Other reported symptoms included weight loss (57.5%) and weakness (90%). Fever and night sweating was also reported by some. 95% people who were tested positive depended on government hospitals and health centres for treatment and medicine. Only a very few consulted private doctors or clinics. Many people had to travel long distances to reach government hospitals.

The major difference noted between patients who visited government hospitals and private hospitals was the lack of knowledge and awareness they had about TB and its consequences. The people who consulted government centres were unaware about the type of TB, their HIV status and also about the kind of diet supplements like fruits and vitamins that they should be taking. Whereas the people visiting private hospitals for their treatment responded well about their condition and the diet that they were following.

But the main reason for people to depend on government hospitals is the free treatment including consultation and medicines. Because most of the people were from financially backward category which gave them no option other than to rely on government services for such treatments. Government centres should also include more awareness to people in their treatment method which can make people conscious about the condition and also educate other people around them.

The disease has made people either to quit their jobs or to depend on other family members for their

living. Most of the people belonged to BPL category and only earned 300 – 500rs per day initially. After the pandemic, since many people lost their jobs and many couldn't work, made the condition more miserable for the patient and their family to live.

Majority of the patients were male (Table I), and were the primary income earners in their household. Also 50% of the cases belonged to 21-40 age group where young men were forced to leave their jobs and married men had to survive and look after their children without any income.

Lack of awareness has very major role in the poor outcomes reported. People living in a 5 minutes walking distance from the hospital has also not consulted any doctor with history of one year of cough and weight loss. Such situations can only be avoided in the future with frequent awareness and increased screening for TB which should be arranged by all the hospitals together.

Discussion

Despite advances in care and diagnosis, tuberculosis remains a major public health concern, particularly in developing countries. Even though tuberculosis is an avoidable disease, the alarming news is that the number of new tuberculosis cases has not dropped. In India, tuberculosis has been a serious public health issue with negative social and economic effects.[8] The rise of antibiotic resistant strains, particularly MDR TB, has exacerbated the problem. Increased HIV infection, inadequate tuberculosis management, and laboratory inefficiencies in diagnosing M.Tuberculosis are all contributing causes to the recent MDR TB outbreak and its continued growth..[9] The fast and accurate identification of infected persons is critical to its control. Microscopy detection of acid-fast bacilli is the simplest rapid diagnostic approach. The gold standard for diagnosing pulmonary tuberculosis is a conventional culture, although it takes several weeks to turn positive. As a result, a faster, less expensive, and more effective approach has been devised.[10] The current study focused on active pulmonary tuberculosis with a positive direct smear, whereas prior investigations included both negative and positive direct smear cases. The results were based on four sets of RSC. One on the 3rd day, one on the 6th day, one on the 9th day, and one on the 12th day, respectively. These extra processes can also improve the RSC's sensitivity as compared to other media such as LJ. The translucent Middle Brook 7H9 medium employed in this work is fairly inexpensive, as is the malachite green utilised to prevent contamination. RSC is more sensitive and specific for Mycobacterium TB growth than many other studies, which makes it more sensitive and specific for *Mycobacterium tuberculosis* growth. In the current trial, RSC took 6-12 days to complete. Rapid slide culture has the advantage of allowing colonies to be viewed without the need of an inverted microscope, and results can be obtained in as little as seven days. Rapid slide culture has proven to be a quick, low-cost, and effective way to identify tuberculosis.[12] When it comes to examining the poor outcomes of people with pulmonary tuberculosis, those who had a poor understanding of the disease had a higher probability of having a poor treatment outcome than those who had a strong understanding.[13] Fatiregun AA et al[11] also reported this observation. The bulk of the patients in the current study were men. It could be due to the fact that women from lesser socioeconomic and educational backgrounds do not prioritise their health, or it could be due to the fact that women's health is undervalued. Men who are financially self-sufficient are given preferential treatment, whereas women who rely on other family members for financial support are often neglected. As a result, while the study included more men, it is impossible to establish that men are more afflicted.[14] Knowing the elements that influence treatment success will lead to the implementation of specific PTB management techniques that may help to reduce treatment failure.[12] Unsuccessful treatment outcomes could also be due to other circumstances, such as the patient's age, unemployment, and the financial hardship of continuing therapy, or a patient from a bigger family who is responsible for the entire home. In this study, several such situations were encountered, and comparable findings were also reported by Tekkel M etal[13]. Low income is linked to unemployment and bigger family sizes. Malnutrition is common among low-income patients, which can lead to increased treatment side effects and decreased stamina, as well as poor adherence, mortality, or abandonment of anti-TB chemotherapy. Poverty was identified as one of the key risk factors for tuberculosis in a study conducted by Belo MTC etal[14] in Brazil. The majority of TB patients in our study came from low-income families. Another study, which agreed with this one, found that unemployment was strongly linked to a poor treatment outcome.[15]

Conclusion

When compared to other culture methods, the current study produced good RSC findings. RSC was only slightly more expensive than culture in L-J medium, and it had a significant advantage over both L-J and MGIT in terms of turnaround time. As a result, RSC should be further investigated to see if it can meet the great desire for a quick, sensitive, and low-cost culture approach in resource-constrained environments. The strength of this study is its capacity to gather verifiable data from TB patients in order to establish treatment outcomes. We were able to generalise our findings after studying 40 patients at random. Older age, larger family sizes, unemployment, and a lower educational background were identified as predictors of failed treatment outcomes. Following this finding, we recommend that patients who are at high risk of a poor treatment result be identified early and provided more follow-up, as well as a combination of medical care and social support.

Ethical Clearence: This current study is approved by the Institutional ethics committee of JSS Medical college and Hospital, JSSAHER, Mysore.

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