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Original Research Article.

The Effectiveness of Microscopy and Culture in the Detection of Mycobacterium Tuberculosis Among the Smear Negative Pulmonary and Extra Pulmonary Tuberculosis

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ABSTRACT

Background: TB is still a lingering global threat. India falls under the high burden label. The present study is undertaken to compare the effectiveness of microscopy and culture in the detection of Mycobacterium tuberculosis among the smear negative pulmonary and extra pulmonary tuberculosis.

Materials and Methods: The present study was conducted to evaluate the effectiveness of microscopy and culture in the detection of Mycobacterium tuberculosis among the smear negative pulmonary and extra pulmonary tuberculosis. A total number of 200 patients were included in the study. Sputum smear negative pulmonary and extra pulmonary specimens were subjected to direct microscopy by Zeihl-Nielsen and fluorescent staining. Culture was done by Rapid slide culture, Lowenstein Jensen media (LJ media) and BACTEC MGIT system.

Results: Radiological study was suggestive of TB in 75% cases and negative in 25% cases. Of total 200 cases, 60% cases were pulmonary samples and 40% were extrapulmonary samples. 4% cases were positive, and 96% cases were negative with ZN stain and fluorescent stain on direct smear. After concentration 5% cases were found to be positive. In rapid/Modified slide culture method, 5% cases were found to be positive and 95% were negative. In LJ medium, 8% cases were positive, and 92% cases were negative. 20% cases were found to be positive for growth and 80% cases were found negative for growth in MGIT method.

Conclusion: The present study concluded that sensitivity of direct microscopy was 4% and after concentration it was 5%. Overall culture positivity was 20%. Detection rate by LJ method, rapid slide culture method and MGIT methods was 8%, 5% and 20% respectively.

Keywords: ZN Stain, Tuberculosis, LJ Medium.

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INTRODUCTION

Pulmonary tuberculosis (PTB) is a bacterial infection caused by M. tuberculosis, spread by inhaling droplets of mucus that have been expelled by an infected person. Globally PTB is the leading cause of death among infections, killing 3 million people every year. Tuberculosis is India's worst scourge as it bears one-third of the entire world's tuberculosis burden.[1] The rapid diagnosis of TB is central to minimizing the risk of disease transmission, especially in the wake of the emergence of drug-resistant TB and its severe implications for human immunodeficiency virus-infected patients.[2] Clinicians evaluate patients with suspected TB by medical history, physical examination, chest radiograph and checking up on patiens symptoms. TB is diagnosed by detecting of Mtb bacteria in a clinical specimen. Culture is remaining the gold standard for laboratory confirmation of TB disease, and growing bacteria are required to perform drug-susceptibility testing. GeneXpert MTB/RIF (GX) (Cepheid, Sunnyvale, California, USA) assay is a new molecular test for TB which diagnoses Mtb by detecting the presence of Mtb bacteria, as well as testing for resistance to the drug rifampin.[2,3] Smear microscopy results can be obtained within 2 h; however, smear microscopy is less sensitive because it requires 5000–10,000 bacilli per mL of sputum for showing a positive result. Almost 13% of TB transmission occurs with smear-negative, culture-positive TB patients. Therefore, healthy individuals are at risk of MTB infection leading to active TB development when coming in close contact with sputum-negative TB suspects. Moreover, this test requires 3-day early morning sputum specimen collection protocol to enhance sensitivity. In addition to the lower sensitivity of sputum smear microscopy, it

cannot differentiate MTB from MTB complex.[4] Therefore the present study is undertaken to compare the effectiveness of microscopy and culture in the detection of Mycobacterium tuberculosis among the smear negative pulmonary and extra pulmonary tuberculosis.

MATERIALS AND METHODS

The present study was conducted to evaluate the effectiveness of microscopy and culture in the detection of Mycobacterium tuberculosis among the smear negative pulmonary and extra pulmonary tuberculosis. Before the commencement of the study ethical approval was taken from the ethical committee of the institute.

The present study included the specimens from the following patients: 1. Patients having radiological lesions suggestive of active pulmonary/ extra pulmonary tuberculosis. 2. Patients having strong clinical evidence suggestive of extra pulmonary Tuberculosis and 3. Sputum smear negative for M. tuberculosis among clinically suspected pulmonary TB cases. Direct smear was prepared from the purulent portion of the specimen on a clean slide on an area of 2 × 1 cm. They were air-dried, heat fixed and subjected to Zeihl-Nielsen staining (ZN stain) and fluorescent staining as recommended by National Tuberculosis Institute. Smears were graded according to RNTCP guidelines.[5] Smears were also made after concentration and subjected for ZN and fluorescent staining. From the centrifuged sediment, smear was done over the lower one-third of two slides using one half of the longitudinally cut slide. The slides were put inside a Mc cartney bottle with the smear immersed in a medium containing seven ml of citrated human blood diluted with deionized water and made selective with addition of Polymyxin B (2,00,000 units/L), carbenicillin (100mg/L), Trimethoprim (10mg/L) and Amphotericin B (10 mg/dL). The inoculated medium was incubated for seven days at 37°C. On the seventh day the slides were dipped in sterile water, dried in hot air oven at 80°C for 30 minutes, stained by ZN method and fluorescent method. Slides were then examined under oil immersion for microcolonies of acid-fast bacilli. The growth was recorded and graded. From the sediment, one loopful each was inoculated on to two slopes of Lowenstein-Jensen medium using a 5 micron, 22 guage, nichrome wire loop. Date of inoculation was noted. The slopes were incubated at 37°C for a maximum period of eight weeks. The slopes were inspected daily for the growth or for contamination. In case of growth of Mycobacteria, date of appearance of first colony was noted and slopes were further incubated for more growth. Culture in Mycobacterium growth indicator tube (MGIT) by semiautomated methods: Protocol of Ruhi Bunger et al was followed.8 Becton Dickinson (BD) BBL MGIT tubes were used for processing and the BACTEC MGIT TB system Product and Procedure manual was referred to. MICRO-MGIT system was used to look for green signal to indicate growth in the tube. Visually the Mycobacterium growth appears granular white deposit at the bottom of the tube with clear liquid above. Growth of Mycobacterium was confirmed by aseptically pipetting one or two drops of media and preparing smears which were stained by ZN and fluorescent method. A drop of the fluid was also inoculated onto 5% sheep blood agar in order to rule out contamination. Specimens positive for Acid fast bacilli in smear and showing no growth on 5% sheep blood agar were considered positive for Mycobacterial growth. Immunochromatography assay to detect MPT64 antigen using SD BIOLINE TBAg MPT64 rapid antigen detection kit was performed to confirm the positive isolates of M. tuberculosis complex and to differentiate from Nontuberculous mycobacteria. Results were tabulated and statistically analysed.

Table 1: Correlation of direct smear finding, concentration method, rapid slide culture and LJ medium with MGIT method

Method	Criteria	No. of patients	MGIT Method	
			Positive (20%)	Negative (80%)
Direct smear method	**Positive**	8(4%)	10(25%)	0
	Negative	192(96%)	30(75%)	160(100%)
Concentration method	**Positive**	10(5%)	14(35%)	0
	Negative	190(95%)	26(65%)	160(100%)
Rapid Slide Culture	**Positive**	10(5%)	14(35%)	0
	Negative	190(95%)	26(65%)	160(100%)
LJ method	**Positive**	16(8%)	20(50%)	0
	Negative	184(92%)	20(50%)	160(100%)

RESULTS

A total number of 200 patients were included in the study. Radiological study was suggestive of TB in 75% cases and negative in 25% cases. Of total 200 cases, 60% cases were pulmonary samples and 40% were extrapulmonary samples. Table 1 shows correlation of direct smear finding, concentration method, rapid slide culture and LJ medium with MGIT method. 4% cases were positive, and 96% cases were negative with ZN stain and fluorescent stain on direct smear. After concentration 5% cases were found to be positive. In rapid/Modified slide culture method, 5% cases were found to be positive and 95% were negative. In LJ medium, 8% cases were positive, and 92% cases were negative. 20% cases were found to be positive for growth and 80% cases were found negative for growth in MGIT method.

DISCUSSION

Early TB diagnosis is essential for interrupting the transmission chain of TB disease. It is well known that AFB smear microscopy–positive TB patients are the major source of spreading TB to healthy individuals when left untreated. The studies also reported that approximately 17% of TB disease transmission is caused by AFB smear microscopy–negative TB suspects and therefore the risk of disease transmission by AFB-negative cases to healthy individuals could not be ignored.[6] The continuously increasing frequency of TB in developing countries is a major threat to the life of human being and requires the use of highly sensitive and specific techniques for early detection of MTB.[7]

A total number of 200 patients were included in the study. Radiological study was suggestive of TB in 75% cases and

negative in 25% cases. Of total 200 cases, 60% cases were pulmonary samples and 40% were extrapulmonary samples. 4% cases were positive, and 96% cases were negative with ZN stain and fluorescent stain on direct smear. After concentration 5% cases were found to be positive. In rapid/Modified slide culture method, 5% cases were found to be positive and 95% were negative. In LJ medium, 8% cases were positive, and 92% cases were negative. 20% cases were found to be positive for growth and 80% cases were found negative for growth in MGIT method.

In countries with a high incidence of TB, microscopic examination of sputum smear samples is often the only available diagnostic test for TB. As a result, patients with smear-negative TB do not receive a diagnosis in a timely manner; thus, disease may further develop, initiation of treatment may be delayed, and further TB transmission may occur.[8] Bunger at el and Sarada et al who reported a direct smear positivity rate of 8.33% and 7.69% respectively among extrapulmonary samples.[9,10]

Bunger et al and Jagadish Rawat et al which show a higher percentage of positivity with MGIT method as compared to LJ method and higher rate of sensitivity in case of smear negative samples. Hence, clearly MGIT is a better method of culture as compared to LJ method.[9,11]

CONCLUSION

The present study concluded that sensitivity of direct microscopy was 4% and after concentration it was 5%. Overall culture positivity was 20%. Detection rate by LJ method, rapid slide culture method and MGIT methods was 8%, 5% and 20% respectively**.**

REFERENCES

1. Thorpe LE and Frieden TR. Seasonality of TB in India, Is it real and what does it tell us? The Lancet 2004; 364(9445):1613-14.

2. Tuberculosis Control India.http://www.tbcindia.org.

3. Afsar I, Afsar FS. Evaluation of laboratory diagnosis for cutaneous tuberculosis. Indian J Pathol Microbiol. 2016: 59 (3); 274-8.PMID. 27510659.

4. Helb D, Jones M, Story E, Boehme C, Wallace E, Ho K et al. Rapid detection of Mycobacterium tuberculosis and rifampin resistance by use of on-demand, near-patient technology. J Clin Microbiol. 2010: 48 (1): 229-37. PMID.19864480.

5. Caulfield AJ, Wengenack NL. (2016) Diagnosis of active tuberculosis disease: From microscopy to molecular techniques. Journal of Clinical Tuberculosis and Other Mycobacterial Diseases 4: 33–43.

6. Keflie TS, Ameni G. (2014) Microscopic examination and smear negative pulmonary tuberculosis in Ethiopia. The Pan African Medical Journal 19: 162.

7. Rasool G, Riaz M, Mahmood Z, et al. (2018) Effects of household bleach on sputum smear microscopy to concentrate acid fast bacilli for the diagnosis of pulmonary tuberculosis. Journal of Biological Regulators and Homeostatic Agents 32(3): 607–611.

8. Siddiqi K, Lambert ML, WalleyJ. Clinical diagnosis of smear-negative pulmonary tuberculosis in low-income countries: the current evidence, Lancet Infect Dis, 2003 (3):288-96.

9. Bunger R, Singh V.A, Avneet, Mehta S and Pathania D. Evaluation of BACTEC Micro MGIT with Lowenstein Jensen media for detection of Mycobacteria in clinically Suspected patients of Extra pulmonary Tuberculosis in a tertiary care Hospital at Mullana (Ambala). Journal of Medical Microbiology and Diagnosis. 2013:2(2).

10. Sarada Dasari, Suguneswari Giddi, Aarthi Vara, Sisira D. Smear microscopy and conventional culture on Lowenstein – Jensen media in cases of Tuberculosis - A comparative study. Indian Journal of Microbiology Research. 2017; 4(2): 203-206.

11. Rawat J, Biswas D, Sindhwani G, Masih V and Chauhan B.S. Diagnostic role of MGIT culture of BAL samples in sputum smear negative pulmonary tuberculosis. Indian Journal of Tuberculosis. 2013;77-82.

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