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Research Article

Detection of *Mycobacterium Tuberculosis* (MTB) in Pulmonary Samples by AFB Smear and LJ Culture

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ABSTRACT

Tuberculosis (TB) is a major air-borne infectious bacterial disease. Despite the downward trend of TB, it continues to be a challenging public health problem for its emergent morbidity and mortality worldwide, especially in developing countries. In 2013, nine million people with TB infection estimated by the World Health Organization (WHO), and 1.7 million died from the disease. Ziehl-Neelsen (ZN) smear is widely used for rapid diagnosis of tuberculosis in our country and other developing countries but culture on Lowenstein – Jensen (LJ) medium is more sensitive and cheaper than modern diagnostic techniques in the present settings. This comparative study was carried out in the Department of Microbiology, National Institute of Medical Sciences, Jaipur Rajasthan from January 2019 to June 2019. Study subjects included patient visiting TB OPD clinic and wards of NIMS hospital. A total 240 specimen were processed for both smear & culture. Smear stained with ZN method and culture inoculated on LJ medium after digestion and decontamination of clinical specimen. Out of 240 specimen, 34 (14.1%) were smear positive while 43 (17.9%) were culture positive. Out of 34 smears positive pulmonary tuberculosis cases 29 (85.2%) were found to be positive on LJ culture. A total of 14 (4.8%) pulmonary cases were negative on the smear but found to be positive on LJ culture for *Mycobacterium tuberculosis*. Although AFB smear is rapid, cheap and specific test for early diagnosis of tuberculosis, but its sensitivity is low and culture medium is still a gold standard method although it takes longer time to grow and provide us positive growth to do drug susceptibility testing.

Key words: Acid Fast Bacilli, Ziehl-Neelsen, Lowenstein – Jensen, *Mycobacterium tuberculosis*.

INTRODUCTION

Tuberculosis (TB) is a major air-borne infectious bacterial disease. Despite the downward trend of TB, it continues to be a challenging public health problem for its emergent morbidity and mortality worldwide, especially in developing countries [1]. In 2013, nine million people with TB infection estimated by the World Health Organization (WHO), and 1.7 million died from the disease [2]. The diagnosis of tuberculosis infection is vital both clinically and epidemiologically4. A presumptive diagnosis is crucial to guide treatment, to limit the person to person spread of the disease and to assess the degree of activity of the disease [3].

Acid-fast bacilli (AFB) microscopy, which is a means of detecting/screening of pulmonary tuberculosis, has been used worldwide as a mainstay of case finding [4]. Ziehl Neelsen staining is a cheap and specific test which takes about 1 to 2 hours for reporting; however, it is less sensitive and requires a large number of bacilli (up to 10,000 bacilli/ml) in the specimen. Moreover, it cannot distinguish *Mycobacterium tuberculosis* from *Mycobacterium* other than tuberculosis and is therefore, used for screening only. This technique is widely used in developing countries like India and other [5].

Culture on Lowenstein Jensen (LJ) medium remains the gold standard for the diagnosis of tuberculosis however the facility is not available on its full extent in developing countries [7] hence required special procedures and need skilled technicians. Culture on LJ medium is time consuming but cheaper than radiometric and molecular based techniques and a handy approach in the diagnosis of TB. This study has been conducted to analyze and evaluate the validity and reliability of sputum smear direct microscopy and its contribution in the ultimate diagnosis of PTB [8].

MATERIAL AND METHODS

This comparative study was carried out in the Department of Microbiology, National Institute of Medical Sciences Jaipur from June January 2019 to June 2019. Study subjects included patient visiting TB OPD clinic and wards of NIMS hospital.

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Inclusion Criteria

- Patients of all age group and both genders.
- Newly clinical suspected cases of Pulmonary TB, patients underwent ZN stain, and confirmed with LJ culture.

Exclusion Criteria

- Patients who had received anti-tuberculosis treatment in the previous months.
- Samples suggestive of saliva.
- Contaminated samples.
- Samples rapidly diagnosed by ZN, but confirmed with PCR instead of LJ.
- Any other sample collected instead of sputum (i.e. body fluid samples).

Sample Size

A total 240 sputum specimen were processed for both smear & culture. Smear stained with ZN method and culture inoculated on LJ medium after digestion and decontamination of clinical specimen.

Laboratory Methods

Sample Processing

Early morning sputum specimen of all the patients was collected in sterile container. The preparation of smear and staining and culture was done as per RNTCP guidelines. Direct and concentrated smears of sputum sample were prepared from clinical specimens after treating with 4 % NaOH (sodium hydroxide) for decontamination and digestion of clinical specimens. Sterile phosphate buffer PH 6.8 is added to neutralize the effect of NaOH and the samples were concentrated by centrifugation at 3000 rpm for 15 minutes. Supernatant was discarded and sediment was re-suspended in small amount (1-2 ml) of phosphate buffer and inoculated on the slants of LJ medium. The smears were stained with ZN method using 1% Carbolfuchsin, 20% sulphuric acid and 0.3% methylene blue. A minimum of 100 oil immersion fields was observed to declare negative smear. The grading of sputum smears was done as per RNTCP guidelines for ZN staining [9].

RESULTS

Out of 240 specimen, 34 (14.1%) were smear positive while 43 (17.9%) were culture positive. Out of 34 smears positive pulmonary tuberculosis cases 29 (85.2%) were found to be positive on LJ culture. A total of 14 (4.8%) pulmonary cases were negative on the smear but found to be positive on LJ culture for *Mycobacterium tuberculosis*.

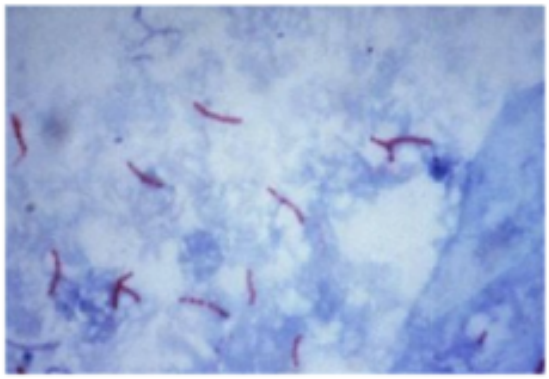

Figure 1: Acid Fast Bacilli (AFB) in ZN staining (100X Oil Immersion)

Figure 2: *Mycobacterium tuberculosis* growth on L-J Medium

Table 1: Comparison of AFB smear with culture results in 240 pulmonary cases

Nature of Specimen	Smear Positive	Culture Positive	Smear Positive / Culture Positive	Smear Negative / Culture Positive
Sputum (240)	34 (14.1%)	43 (17.9%)	29 (85.2%)	14 (4.8%)

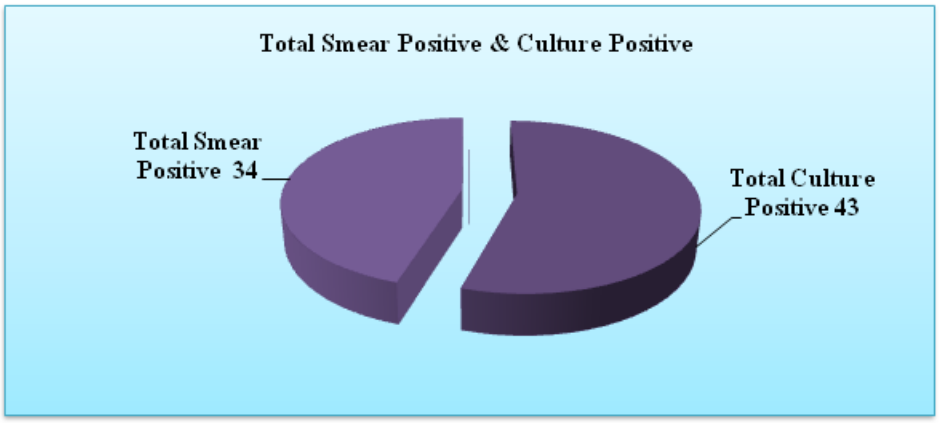

Figure 3:

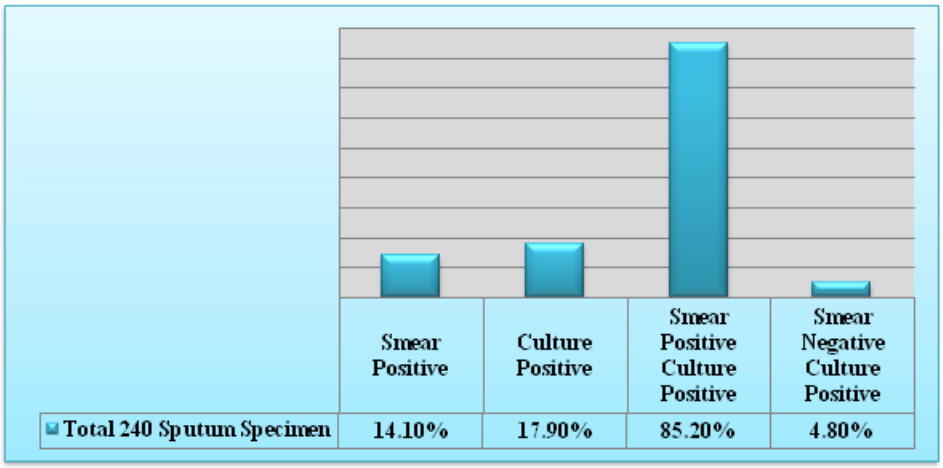

Figure 4:

DISCUSSION

Acid-fast microscopy is believed to be the most practical and fastest technique in establishing a presumptive diagnosis of pulmonary tuberculosis. The sensitivity and specificity of AFB smear microscopy and culture varies depending upon the nature of the specimen, its quality, quantity, bacterial content and the extent of viable organisms [10] Various factors influence the sensitivity and specificity of direct microscopy for AFB (Acid Fast Bacilli), like prevalence and severity of the disease, the quality of specimen collection, the number of mycobacteria present in the specimen, the method of processing (Direct or concentrated), the staining technique and the quality of the examination (Microscope operator expertise, time spent for smear examination) etc [11] [12]. The reasons for huge variability in sensitivity and specificity in various studies are also multiple. Methodology is very important as the sensitivity would be different if different smears are prepared for staining with ZN method. In our study we tried to address most of these issues. The gold standard in our study is most reliable i.e. culture and only 0.5% of the cultures were contaminated. Overall Smear positivity of AFB for pulmonary specimen in the present study is 14.1% and it is little low as compared with International Union against Tuberculosis and Lung Disease (IUTALD) [13]. Smear positivity of pulmonary specimens is (14.1%) and this study is not in agreement with the study that reported the smear positivity of 20.25% in pulmonary specimens [14].

Culture using LJ medium has been the gold standard for the diagnosis of tuberculosis for many years in the developing countries [15]. An overall AFB culture positivity in the present study was 15.47% and is little higher than the study that revealed the culture positivity of 17.9%.While others have reported a culture positivity of 48.9% and 47.1% respectively [16, 17].Culture positivity in the present study is significantly high as compared to AFB smear microscopy as about 5000 to 10000 AFB/ml of specimen is needed to yield positive result by AFB smear microscopy while the advantage of culture on LJ medium is that it has the sensitivity of 80-85%, very specific and being able to detect as few as 10 bacteria per millilitre of specimen [18,19].

CONCLUSIONS

Positivity of cultures in this study is significantly high in comparison to microscopy of AFB smears. Positive result by AFB smear microscopy as about 5000 – 10000 AFB/ml of specimen is needed while about culture on LJ medium the advantage is that it can detect as few as 10 bacteria per millilitres of specimen. The study concludes that although AFB smear is rapid, cheap and specific test for early diagnosis of tuberculosis but its sensitivity is low. It is thus evident that culture on LJ medium is more sensitive and is documented to be gold standard although it takes longer time to grow and provide us positive growth to do drug susceptibility testing. It is cost effective than radiometric and molecular methods and can therefore be a useful tool for developing countries. It is also suggested that facilities for carrying out culture of mycobacterium tuberculosis should be made available at individual level hospitals, especially in Primary Health Centres (PHC) over the country where Cartridge Based Nucleic Acid Amplification Technique (CBNAAT) or TrueNat is not available as CBNAAT and TrueNat is available at tertiary care centres only.

REFERENCES

1. MafijUddin MK, Chowdhury MR, Ahmed S, Rahman MT, Khatun R, Leth FV, et al. Comparison of direct versus concentrated smear microscopy in detection of pulmonary tuberculosis; BMC Research Notes 2013, 6:291.
2. Iqbal S, Iqbal R, Khan MM, Hussain I, Akhtar A, Shabbir I. Comparison of two conventional techniques used for the diagnosis of tuberculosis cases. Int J AgriBioi. 2003; 5(4): 545-7.
3. Kumar NS, Sekar MG, Kumar V, Rao DVB, Rahman F, Narayanan PR. Sensitivity of Ziehl- Neelsen method for centrifuged deposit samples transported in cetyl-pyridinium chloride. Indian J Med Res. 2006; 124:439-42.
4. Aftab R, Amjad F, Khurshid R, Ahmed N. Detection of Mycobacterium tuberculosis in clinical samples by smear and culture. Rav J Med Res. 2008; 1:1-5.
5. Tuberculosis Division International Union against Tuberculosis and Lung Disease. Tuberculosis Bacteriology—Priorities and Indications in High Prevalence Countries: Position of the Technical Staff of the Tuberculosis Division of the International Union against Tuberculosis and Lung Disease. International Journal of Tuberculosis and Lung Disease, 2005;9: 355-361.
6. World Health Organization. WHO Laboratory Services in Tuberculosis Control. Part II: Microscopy. WHO/TB/98.258. WHO, Geneva, 1998;30-32.
7. Shinnick, T.M. and Good, R.C. Diagnostic Mycobacteriology Laboratory Practices. Clinical Infectious Diseases, 1995; 21: 291-299.
8. Smithwick, R.W. Laboratory Manual for Acid-Fast Microscopy. 2nd Edition, US Public Health Service, Atlanta. 1976.
9. Government of India, Central TB Division. Managing the Revised National Tuberculosis Control Programme in Your Area. Directorate General of Health Services. Ministry of Health and Family Welfare, New Delhi. 2005.
10. Masood, Z., Mohammad, N., Majid, K. and Ghodsieh, A. Comparison of the Value of Two Different Sputum Staining for Diagnosis of Acid-Fast Bacilli. Iranian Journal of Clinical Infectious Diseases, 2008;3: 99-102.
11. Negi SS, Khan SFB, Gupta S, Pasha ST, Kare S, Lal S. Comparison of the conventional diagnostic modalities, BACTEC culture and polymerase chain reaction test for diagnosis of tuberculosis. Indian J Med Microbiol. 2005; 23:29-33.
12. Enarson 0A, Rieder HL, Arnadottir T et al. Technical Guide; Sputum Examination fortuberculosis by direct microscopy in low-income countries. 5th ed. Paris: IUATLD, 2000.
13. Kumar NS, Sekar MG, Kumar V, Rao DVB, Rahman F, Narayanan PR. Sensitivity of Ziehl- Neelsen method for centrifuged deposit samples transported in cetyl-pyridinium chloride. Indian J Med Res. 2006; 124:439-42.
14. Boyd, J.C. and Marr, J.J. Decreasing Reliability of Acid-Fast Smear Techniques for Detection of Tuberculosis. Annals of Internal Medicine, 1975; 82: 489-492.
15. Masood, Z., Mohammad, N., Majid, K. and Ghodsieh, A. Comparison of the Value of Two Different Sputum Staining for

Diagnosis of Acid-Fast Bacilli. Iranian Journal of Clinical Infectious Diseases, 2008; 3: 99-102.

16. Trusov, A., Bumgarner, R., Valijev, R., Chestnova, R., Talevski, S., Vragoterova, C. and Neeley, E.S. Comparison of Lumin™ LED Fluorescent Attachment, Fluorescent Microscopy and Ziehl-Neelsen for AFB Diagnosis. International Journal of Tuberculosis and Lung Disease, 2009; 13: 836-841.
17. Bansal, R., Sharma, P.K., Jaryal, S.C., Gupta, P.K. and Kumar, D. Comparison of Sensitivity and Specificity of ZN and Fluorescent Stain Microscopy with Culture as Gold Standard. Journal of Tuberculosis Research, 2017; 5:118-128.
18. Aftab R, Amjad F, Khurshid R, Ahmed N. Detection of Mycobacterium tuberculosis in clinical samples by smear and culture. Rav J Med Res. 2008; 1:1-5.
19. Peter Daley P, Michael JS, Kalaiselvan S, Latha A, Mathai D, John KR, MadhukarPaL. A Pilot Study of Short-Duration Sputum Pretreatment Procedures for Optimizing Smear Microscopy for Tuberculosis. PLoS ONE. 2009;4(5): e5626.