Research Paper Medical Science

Detection of *Mycobacterium Tuberculosis* in Pulmonary Tuberculosis Patients by ZN Smear and Culture

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ABSTRACT

Tuberculosis is a chronic, communicable disease caused by tubercle bacilli. Early diagnosis is an important aspect to control TB. Diagnosis of TB is dependent on the microscopic demonstration of AFB and identification of M. tuberculosis by solid culture methods. This study was carried out on 196 suspected Pulmonary TB patients. All the specimens were subjected to smear microscopy and mycobacterial culture on L-J medium. All 196 Sputum sample were received in the TB laboratory from out and in patients JNMC&H, AMU, Aligarh. Out of 196 suspected patients , majority complained of low grade fever 176 (89.7%), 138 (70.4%) presented with cough with sputum for more than three weeks and 130 (66.3%) had weight loss. The other complaints were shortness of breath 45 (22.9%) and cervical lymphadenopathy 16 (8.1%). Out of 196 suspected cases; 38(19.3%) were ZN Smear positive and 92(46.9%) were culture positive. The aim of this study was to compare the smear stained by ZN method and growth on Lowenstein–Jensen medium for the detection of M. tuberculosis in PTB patients.

Keywords : Pulmonary tuberculosis (PTB), mycobacterial culture, ZN Smear, *M.tuberculosis.*

1. Introduction:

Tuberculosis (TB) remains a major global health problem. It causes ill-health among millions of people each year and ranks as the second leading cause of death from an infectious disease worldwide, after the human immunodeficiency virus (HIV). According to latest World TB report of WHO, In 2011, there were an estimated 8.7 million new cases of TB, equivalent to 125 cases per 100 000 population. Geographically, India and China together account for almost 40% of the World's TB cases (WHO 2012). Early diagnosis is an important aspect in TB control. The conventional method for diagnosing TB using clinical samples by the AFB smear has low sensitivity and specificity. Under the RNTCP guidelines laboratory diagnosis of TB relies on microscopic examination of smears (B.Nandgopal et al 2010). Smear microscopy although rapid and inexpensive but lacks sensitivity. It can detect AFB, if the smear contains ≥ 1000 bacilli which mean that specimen must have ≥ 10,000 bacilli per ml (Yeager et al., 1967). AFB culture is gold standard and sensitive (10-100 viable bacilli are required). ZN smear provide preliminary diagnostic information within an hour suffer from several limitation such as low sensitivity and specificity especially in pulmonary tuberculosis. Serology and other newer techniques are not widely used due to high cost, low sensitivity and specificity or both and use of PCR in clinical practice in developing countries is not very well evaluated for its validity and reproducibility in detection of *M. tuberculosis* in clinical specimens (I Shukla et al 2011). The aim of the present study was to evaluate sensitivity and specificity of culture diagnosis of pulmonary tuberculosis and also compare the results of conventional ZN stained acid fast bacilli microscopy and culture on L-J medium.

2. Materials & methods:

The present study was carried out on 196 patients attending outpatient and inpatient departments of JNMC, AMU, Aligarh. Who were suspected to be suffering from pulmonary tuberculosis. The patients were advised to collect 4 to 5 ml of early morning sputum in a sterilized container and they were instructed to rinse their mouth with pure water and clean their teeth before collection to avoid contamination with food and other particles. The two consecutive days sputum samples were collected as per RNTCP criteria. One spot sputum specimen when the patient first was attends the hospital and another next day morning specimen. A detailed questionnaire and consent was obtained from each patient. The sputum specimens were processed by standard method, one portion of the sputum was subjected to routine microscopic examination using Ziehl Neelsen method and rest of the sputum was digested and decontamination by modified Petroff's method and concentrated by centrifugation at 3000g for 20 minutes, the two Lowenstein Jensen's medium slant were inoculated and were incubated at 37°C for 6-8 weeks. The inoculated L-J media were examined every second day during the first week and then weekly for upto 8 weeks for presence of growth. The growth was identified by standard morphological and biochemical niacin test.

3. Results & discussion-

Our study included 196 patients with provisional diagnosis of pulmonary tuberculosis attending the outpatient and inpatient departments of JNMC&H Aligarh. Of these suspected PTB cases 124 (63.2%) were male and 72(36.7%) were female patients. Out of 196 suspected patients of pulmonary tuberculosis, majority complained of low grade fever 176 (89.7%), 138 (70.4%) presented cough with sputum for more than three weeks and 130 (66.3%) had weight loss. The other complaints were shortness of breath 45 (22.9%) and cervical lymphadenopathy 16 (8.1%). Out of 196 suspected PTB patients, 92(46.9%) were mycobacterial culture positive and 104(53.1%) negative on L-J medium. Only 38(19.3%) patients were ZN Smear positive (figure 1). All 92 mycobacterial culture positive isolates were identified by both morphologically and biochemically as M.tuberculosis. Out of total 92 M.tuberculosis culture positive sputum specimens, 35(38.1%) were smear positive & 57(61.9%) were smear negative. This low amount of positive results on smear microscopy may be

due to low count of organism, because to detect AFB there should be present 5000-10000 organisms per ml or greater (Iqbal et al 2003, Yeager et al 1967). There were also interesting findings that out of 104 cultures negative sputum specimen, 3(2.8%) were smear positive and 101(97.1%) were smear negative (table 1). This positive detection on smear may be due to false positive as has also been reported in many other studies (Boy & Marr, 1975) or the samples may be collected from the patients, which were on anti-tubercular drugs or the bacteria were not able to grow on the media. In general, direct smear reportedly detects AFB only at concentration of around 10,000 bacilli per ml of the specimen conversely; as few as 100 bacilli per ml may be required for positive culture. Thus culture is the gold standard for diagnosis of mycobacterial infection. The diagnosis can be confirmed, drug susceptibilities can be determined and the treatment response can be monitored with the help of cultures (Vijay Nema 2012). Our study highlights the importance of culturing in tuberculosis, especially in fresh cases. The sensitivity and specificity of AFB smear in our study was 38% & 97.1% respectively. Many other study S. Iqbal et al 2003 & Mendoz et al 1987 reported sensitivity and specificity of smear were 12.1% & 99.1% and12% & 50% respectively. While higher sensitivity & specificity reported by R Aftab et al 2008 & Marie Y et al 1995 were 72.1% & 92% and 79% & 100% respectively.

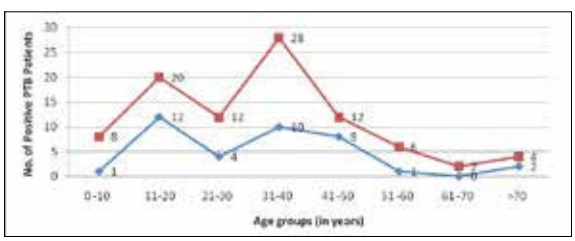

Figure showing the smear and M. tuberculosis culture profile of suspected pulmonary tuberculosis patients. (---smear and --- M. tuberculosis culture positive)

Table I. Sensitivity and specificity of ZN smear microscopy for suspected PTB patients

True positive (both smear & culture positive)	35
False positive (smear positive but Culture negative)	03
True negative (smear negative but culture positive)	57
False negative (both culture and smear negative)	101
Sensitivity	38.04%
Specificity	97.12%

4. Conclusion-

Our study indicated that ZN staining is rapid and inexpensive but lacks sensitivity and specificity. Mycobacterial culture should be a method of choice for the detection of TB cases in spite of its time consuming demerit. The detection of TB cases on molecular level is also in practice in our country but due to the lack of molecular expertise, highly expensive equipment need and expensive, these techniques are not so common. So, it is highly suggested that at least culturing must be recommended and should not be rely only on AFB for the treatment of tuberculosis cases.

REFERENCES

1. WHO Global Tuberculosis Report 2012 | 2. Revised National TB Control Programme Training Manual for Mycobacterium tuberculosis Culture & Drug susceptibility testing, April 2009. | 3. Diagnosis of smear positive pulmonary TB New guidelines, effective from 1st April 2009. | 4. B Nandagopal, S Sankar, K Lingesan, KC Appu, G Sridharan, AK Gopinathan. Evaluation of a nested PCR targeting IS6110 of Mycobacterium tuberculosis for detection of the organism in the leukocyte fraction of blood samples, 2010, (Vol) 28, 3, 227-232 | 5. Sajjad Iqbal, Rizwan Iqbal, M.Mumtaz Khan, Ijaz Hussain, Ajaz Akhtar and Iffat Shabbir. Comparison of two conventional techniques used for the diagnosis of tuberculosis cases, international journal of agriculture & biology, 1560–8530/2003/05–4–545–547 | 6. Boy, J.C. and J. Marr, 1975. Increased threats of TB in new form of MDRTB. Ann. Intern. Med., 82: 489 | 7. Joseph, S., N. G.K. Nair and P.R.J. Gangadharam, 1969. Tuberculosis is still present. Tubercle, 50: 299 | 8. Yeager, H.Jr., J. Lacy, L.R. Smith and C.A. LeMaistre, 1967. Quantitative studies of mycobacterial populations in sputum and saliva. Am. Rev. Respir. Dis., 95: 998–1004 | 9. Vijay Nema, Tuberculosis diagnostics: Challenges and opportunities 2012, lung India, vol.29 (3) 259-266. | 10. Sharma K Surendra, Sanjeev Kumar, PK Saha, Ninoo George, SK Arora, Deepak Gupta, Urvashi Singh, M Hanif, RP Vashisht,Prevalence of multidrug-resistant tuberculosis among category II pulmonary tuberculosis patients, 2011, ijmedical research (Vol)133, 3, 312-315 | 11. Mendoza MT, et al. The predictive value of sputum AFB microscopy. Phil J Microbiol Infect Dis 1987; 17:33-35 | 12. Aftab R, F Amjad and R Khurshid,. Detection of mycobacterium tuberculosis in clinical samples by Pulmonary Tuberculosis in Peshawar smear and culture. Pakistan Journal of Physiology, 2009, 5: 27-30 | 1. Indu Shukla1, Sumit Varshney1, Sarfraz1, Abida Malik1, Zubair Ahmad, Evaluation of nested PCR targeting IS6110 of Mycobacterium tuberculosis for the diagnosis of pulmonary and extra-pulmonary tuberculosis, Biology and Medicine, Vol 3 171-175, 2011.