The Role Of Culture In Diagnosing Smear Negative Tuberculosis In HIV Seropositive Patients.

Umamaheshwari S¹, Sumana MN²
¹Research Scholar, Department of Microbiology, JSS Medical College, JSS University, India.  
²Professor of Microbiology, Department of Microbiology, JSS Medical College, JSS University, India.

Abstract: Sputum microscopy, the most commonly practiced tuberculosis (TB) diagnostic test is less sensitive in immunocompromised patients due to low bacillary load. The study was carried out in a tertiary care general hospital during 2010-12 in Mysore, Karnataka, to diagnose smear negative TB in HIV patients by culture technique. Of 416 HIV patients, 162 patients with features of pulmonary/extrapulmonary TB but smear negative were included in the study. Sputum, stool, blood and other relevant clinical samples except blood were processed as per standard protocol. The blood collected with sodium citrate was processed by lysis centrifugation. All samples were inoculated onto Lowenstein Jensen slants and incubated at 37°C for 6 to 8 weeks. Of 162 HIV patients, 67 (41%) were found to have TB. Extrapulmonary TB (25%) was more common than pulmonary TB (13%). Mycobacteria were recovered in 76 samples (26 sputum, 12 stool, 18 blood, 7 pleural fluid, 1 CSF, 9 FNAC, 1 ascitic fluid, 1 pus, 1 ear discharge). M. tuberculosis (95%) was the predominant species isolated followed by M. avium complex (5%). Most HIV patients with TB are left undiagnosed for reasons of no advanced/high cost techniques in resource constrained settings. Thus culture could be used as a tool in diagnosing smear negative TB.

Keywords: Culture, HIV, Smear negative, Tuberculosis.

I. Introduction

Human Immunodeficiency Virus (HIV) infection and Tuberculosis (TB) increase the rate of disease progression of each other, thereby diminishing the survival time of the patients. Among HIV patients, TB is the most common opportunistic infection that can occur at any stage of HIV infection. The clinical presentation is influenced by the degree of immunosuppression. TB is often atypical in presentation, frequently causing extrapulmonary disease in HIV patients. Diagnosing smear negative TB in resource constrained settings is a challenging task. Sputum microscopy, the most commonly practiced TB diagnostic test in many low income countries detects <50% of TB cases in immunocompromised patients. The sensitivity of this test is less due to absence or reduction in cavitation resulting in low bacillary load in HIV positive patients. HIV infection even diminishes the reliability of chest radiograph in the diagnosis of pulmonary tuberculosis as the disease commonly presents with atypical pattern.[1,2,3] To accelerate the development of TB diagnostics for people with HIV, World Health Organization (WHO) released guidelines in 2006–2007 stating culture of sputum (smear negative) and other clinical samples with a clinical suspicion of TB.[4,5]

Culture of clinical specimen is more sensitive than smear microscopy because 10–100 viable organisms will result in a positive culture whereas 5000–10 000 acid-fast bacilli (AFB) per ml are required for detection by smear.[6] Though BACTEC and other systems are providing the most rapid methods for mycobacterial detection, isolates obtained from cultures can be used for species identification, drug susceptibility and molecular epidemiology.[7, 9] Christie and Callihan suggests that the techniques used to diagnose mycobacterial disease should match with the resources available in the laboratory and the incidence of the disease.[10] Also, the incidence of mycobacteremia is dramatically raised in patients with AIDS. Culture being a gold standard as recommended by WHO, various clinical samples collected from the site of pathology in smear negative patients were cultured to recover Mycobacteria and to assess the usefulness.

II. Materials And Methods

2.1 Study Setting:
The study was conducted at a tertiary care general hospital located in the center of Mysore city, Karnataka, India during Aug 2010 to Dec 2012. It caters to patients coming from Mysore, Chamarajnagar, Mandya, Hassan, Hegaddevankote and Coorg districts.

2.2 Sample Population:
Totally 416 patients were found to be HIV seropositive during the study period and 162 patients with signs and symptoms suggestive of pulmonary/extra pulmonary tuberculosis but smear negative for AFB
constituted the study population. Ethical approval was obtained from the Institutional Ethics Committee, and accordingly informed written consent was obtained from all patients.

2.3 Selection Criteria:
Inclusion Criteria: HIV reactive patients with any of the following features were included as study subjects: a history of prolonged fever, weight loss, cough for more than 2 weeks (sputum smear negative), radiological evidence suggestive of TB, pleural effusion, diarrhoea persisting for>1 month, pain abdomen/ascites/lymphadenopathy or any other features suggestive of TB.
Exclusion Criteria: HIV seronegative patients, HIVseropositive patients with no features of TB or smear positive for AFB and patients with diarrhoea due to parasitic infestation.

2.4 Sample collection and Processing:
A detailed history of each patient was entered in the proforma. Each patient was instructed to provide sputum (if productive) and stool at early morning for AFB smear and culture. Also, blood and other clinical specimens depending on the site of pathology were collected by aseptic methods.

Approximately 1gm of stool sample was emulsified in 5ml of Middlebrook 7H9 broth. The stool and sputum specimens were decontaminated by adding equal amounts of 4% sodium hydroxide (NaOH) and incubated for 20min at 37ºC. All specimens (body fluids if any) except blood were concentrated by centrifugation for 30min at 3000g. Smears of the centrifuged deposit were prepared and stained by Ziehl Neelson (ZN) technique for detection of AFB and inoculated onto Lowstein Jensen (LJ) slant for culture.

Lysis centrifugation technique was followed to process blood samples. Approximately 5 ml of venous blood collected was transferred immediately into 15ml sterile centrifuge tube with 300µl of 3.8% sodium citrate anticoagulant. The tubes were centrifuged at 3000g for 10 min and serum was discarded. The buffycoat was aseptically transferred into Wintrobe tube using a lumbar puncture needle (LP) and centrifuged at 3000g for 30 min. Concentrated buffycoat was transferred using LP needle into a sterile ependoff tube containing 50µl of 0.1% saponin. To enhance the lysis of cells, the mixture was vortexed with coarse glass beads for 10-12 min. From this two smears were prepared and stained by Leishman and ZN stains for the confirmation of cell lysis and presence of AFB respectively. The sample was then inoculated onto LJ medium.

All the LJ slants were incubated at 37ºC for 6 to 8 weeks and were examined for growth twice a week. Any growth observed was confirmed to be Mycobacteria by ZN staining of the culture smear.

III. Results
A total of 162 TB suspected patients out of 416 HIV positive patients were included in the study. The age of these patients ranged from 18 to 97 yrs. The majority were in the age group of 31-40 yrs (70, 43% - Table1). Of 162 patients 124 (77%) were males and 38 (23%) were females.

Totally 76 Mycobacterial isolates were recovered out of 443 clinical samples collected from 162 patients (26 sputum, 12 stool, 18 blood samples, 7 pleural fluid (PF), 1 cerebrospinal fluid (CSF), 9 fine needle aspiration cytology (FNAC) samples, 1 ascitic fluid (AF), 1 pus and 1 ear discharge - Table 2). Out of 162 patients, 67(41%) patients yielded growth of Mycobacteria. In some patients, Mycobacteria were isolated from more than one sample (both sputum and stool in 4 patients, stool and blood in 1 patient, sputum and blood in 1 patient, AF and blood in 1 patient, PF and blood in 1 patient and in FNAC and blood in 1 patient) and in most patients from a single sample (21 from sputum, 7 from stool, 13 from blood, 8 from FNAC, 6 from PF, 1 from pus, 1 from CSF and 1 from ear discharge).

Of the 76 Mycobacterial isolates 69 (95%) were Mycobacterium tuberculosis (MTB) and 7(5%) were Mycobacterium avium. MTB was recovered from 24 sputum, 16 blood, 10 stool, 9 FNAC, 7 pleural fluid, 1 CSF, 1 Ascitic fluid, 1 pus and M.avium from 2 sputum, 2 stool, 2 blood and 1 ear discharge samples (Table 2).

Pulmonary TB was detected in 21(13%) patients and extrapulmonary TB in 41 (25%) patients and 5 (3%) patients had both. Chi Square Test applied to each clinical sample which yielded growth of Mycobacteria showed that pleural fluid (0.394) and FNAC(1.000) were more significant compared to rest of the samples.

<table>
<thead>
<tr>
<th>Age</th>
<th>No. of cases</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-20 yrs</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>21-30 yrs</td>
<td>26</td>
<td>16</td>
</tr>
<tr>
<td>31-40 yrs</td>
<td>70</td>
<td>43</td>
</tr>
<tr>
<td>41-50 yrs</td>
<td>33</td>
<td>20</td>
</tr>
<tr>
<td>51-60 yrs</td>
<td>24</td>
<td>15</td>
</tr>
<tr>
<td>60 yrs above</td>
<td>8</td>
<td>5</td>
</tr>
<tr>
<td>Total</td>
<td>162</td>
<td>100</td>
</tr>
</tbody>
</table>

TABLE 1: Age wise distribution of cases.
The Role Of Culture In Diagnosing Smear Negative Tuberculosis In HIV Seropositive Patients

TABLE 2: Recovery of Mycobacterial isolates from different clinical samples

<table>
<thead>
<tr>
<th>Specimen(s)</th>
<th>Sample No.</th>
<th>Culture positive(%)</th>
<th>M.tuberculosis</th>
<th>M.avium</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood</td>
<td>162</td>
<td>18 (11)</td>
<td>16</td>
<td>2</td>
</tr>
<tr>
<td>Bone</td>
<td>3</td>
<td>0 (0)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Cerebrospinal fluid</td>
<td>20</td>
<td>1 (5)</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>FNAC</td>
<td>22</td>
<td>9 (41)</td>
<td>9</td>
<td>0</td>
</tr>
<tr>
<td>Others (pus, ear discharge,</td>
<td>33</td>
<td>3 (9)</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>skin lesions, ascitic fluid)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pleural fluid</td>
<td>14</td>
<td>7 (50)</td>
<td>7</td>
<td>0</td>
</tr>
<tr>
<td>Sputum</td>
<td>74</td>
<td>26 (35)</td>
<td>24</td>
<td>2</td>
</tr>
<tr>
<td>Stool</td>
<td>115</td>
<td>12 (10)</td>
<td>10</td>
<td>2</td>
</tr>
<tr>
<td>Total</td>
<td>443</td>
<td>76 (35)</td>
<td>69</td>
<td>7</td>
</tr>
</tbody>
</table>

IV Discussion

As per WHO, 5 million people are living with HIV in South, South-East and East Asia.\(^{[11]}\) People with HIV have 20–30 times higher lifetime risk of developing active TB, compared to people without HIV.\(^{[12]}\) In 2010, people living with HIV accounted for about 13% of tuberculosis cases worldwide, and about 3,60,000 died from HIV-related tuberculosis.\(^{[13]}\) Although patients with sputum smear-negative TB are less infectious, they are more likely to die during or before diagnosis than patients with smear-positive TB and contribute to TB transmission.\(^{[14]}^{[15]}\) A follow up study at Malawi reported that smear negative pulmonary TB patients had higher risk of death than smear positive TB with a hazard ratio of 2.2.\(^{[21]}\) Autopsy studies of HIV positive patients identified TB as a cause of death in 14–54% of adults or adolescents.\(^{[17]}^{[19]}\)

Mycobacteremia has now become common in patients with advanced HIV infection and active TB.\(^{[20]}^{[21]}\) Screening of TB among HIV patients with symptoms of fever, cough, weight loss and chest radiography would detect only 25% of HIV-TB co-infected patients.\(^{[22]}\) The culture technique employed in this study detected TB in 67 (41%) out of 162 HIV seropositive, smear negative patients. Out of the 443 clinical samples collected, 76 samples yielded growth of Mycobacterium. Our HIV-TB co-infection rate of 41% concurs with the studies of Zambia -43%,\(^{[23]}\) Pune - 45.3%,\(^{[24]}\) Vadodara, Gujarat - 49.2%,\(^{[25]}\) South African - 49%,\(^{[26]}\) Southern California -43.2%\(^{[27]}\) and Benin, Nigeria -33.9%.\(^{[28]}\) Wide disparity was observed in a Tanzanian study which showed low co-infection rate of 5.3%.\(^{[29]}\) Hence the early TB detection among HIV patients is vital.

In the present study, number of extrapulmonary cases (41-25%) were more than pulmonary cases (21-13%) which concurs with studies of Shimla (25.28% pulmonary TB and 57.47% extrapulmonary TB)\(^{[29]}\) and Vadodara, Gujarat (55% pulmonary and 68% extrapulmonary TB)\(^{[25]}\) but differs from Benin, Nigeria (88% pulmonary and 12% extrapulmonary TB)\(^{[28]}\) and Zambian studies (40% pulmonary, 34% extrapulmonary).\(^{[30]}\)

In the western countries Mycobacterium avium complex (MAC) is the leading cause of TB in HIV infected patients whereas in India, M.tuberculosis is the commonest cause.\(^{[20]}\) In this study MTB (69-95%) was the predominant species isolated followed by M. avium (7-5%) which matches with the study of Buenos Aires wherein MTB was isolated in 223 (92.9%) and MAC in 14 patients (5.8%). The other two species identified in their study were M. kansasiand M. bovis.\(^{[31]}\) Our study differs from studies of Praharaj et al of Pune\(^{[24]}\) who reports only MTB with no atypical Mycobacteria and study of Murcia-Aranguren et al of Bogota, in which M.avium was the predominant species 13(4.2%) and MTB was only 4(1.4%).\(^{[32]}^{[33]}\)

In this study blood culture was found to be positive in 18 (11%) out of 162 samples and 13 patients were found to have TB only by blood culture. Of 18 isolates, MTB was isolated in 16 and MAC from 2. The present study concurs with Truffot-Pernot Cet et al studies where 61 (10.8%) samples taken from 19 patients (11.5%) were positive by blood culture and M. avium intracellulare was the most frequently isolated species.\(^{[33]}\) Studies of M Di Lonardoet al of Buenos Aires differs from this study wherein blood culture was positive in 21.2% which is almost double while Patama Monkongdee et al of Thailand study showed only 2% positivity (16 out of 1051).\(^{[31]}^{[34]}\)

In South African study\(^{[36]}\) 49% of patients who were smear negative showed culture positivity in sputum which slightly differs from our study that shows lower percentage i.e., 26 (35%) out of 74 sputum samples.

Stool culture was positive in 12 (10%) out of 115 samples in the present study which slightly differs from Thailand study where only 61(6%) stool showed culture positivity out of 1052 samples.\(^{[34]}\) Evidence of MTB in the stool may be attributed to gastrointestinal TB or to sputum swallowed by persons with pulmonary TB; in patients with chronic diarrhoea, yield in stool culture may be particularly high.\(^{[35]}^{[37]}\) Patama Monkongdee et al found it a problem to recommend stool culture as an adjunct to diagnose TB because of difficulty in decontamination.\(^{[38]}\) As per our findings though 10% stool were culture positive, very minimal (<1%) contamination rate was observed in the technique we followed.
In our study 7 pleural fluid samples showed growth out of 14 samples which differs from Elliot and Halwiindi et al study which shows pleural fluid culture positivity in 12 (26%) out of 46 patients.[30]

In this study FNAC showed culture positive in 9 (41%) out of 22 samples which concurs with Thailand study (34 out of 82 lymphnode aspirates–42%).[34]

More rapid and more sensitive techniques have replaced traditional methods of direct examination and culturing for diagnosing mycobacterial infections. Among the most recent methods are Isolator blood culture, radiometric detection, hybridization and amplification, each with their own advantages and disadvantages.[38] Also these are costlier compared to conventional culture technique.

Limitation of our study was that a small proportion of TB suspected patients were recognized since it was hospital based and the patients included were those who were seriously ill and hospitalized. Thus, we might have missed patients who were out patient/not seriously ill/not opting to attend the hospital for care.

V. Conclusion

Diagnosis of tuberculosis in PLWH is extremely important in order to plan a therapeutic conduct. The occurrence of TB in HIV accounted for 41% and the extrapulmonary presentation of TB were high in this study. India being a high prevalent area in HIV and TB diseases, physicians should have a high clinical suspicion of TB in HIV patients and all HIV individuals should be screened for TB. If patients are diagnosed at an early stage of disease, the mortality and transmission rate can be reduced as TB is treatable. Culturing sputum and other clinical samples from suspected TB in HIV seropositive cases should be encouraged as part of routine procedure in diagnosing TB. Many patients are left undiagnosed for TB for reasons of no advanced/high cost techniques available. In such situations, existing facilities like culture can be completely utilized. Culture being cost effective, sensitive and a gold standard, can be routinely used in the laboratory as a tool in diagnosing smear negative TB cases.

VI. Acknowledgement

Our sincere thanks to Indian Council of Medical Research (ICMR-IRIS No.5/8/5/8/2008-ECD-I), New Delhi for financial assistance in carrying out this study. We also thank Mr B Mahadevaswamy, Dr K Jayashree, Dr K Anurada, Dr B Sumangala for their valuable inputs for our study.

References:


The Role Of Culture In Diagnosing Smear Negative Tuberculosis In HIV Seropositive Patients


