Research Article

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# Isolation and Identification of *Mycobacterium tuberculosis* from AIDS Patients Attending a Rural Hospital in Central India.

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## Abstract

**Aim:** To isolate and characterize mycobacterium tuberculosis from clinical samples of AIDS patients.

**Materials and Methods:** This cross -sectional observational study was conducted in department of microbiology, in a rural medical college. The inclusion criteria were adult patient of any sex, HIV seropositive, CD4 counts < 200cells/cumm or active TB with CD4 counts>200cells/cumm. Complete blood counts and CD4 counts were done. Blood, sputum, stool and other extra-pulmonary samples were processed for mycobacterial isolation. BACTEC 13A medium was used for blood samples while Lowenstein Jensen and BACTEC 12B medium was used for other samples. Smears were examined for all samples other than blood. Identification of the isolates was done by standard techniques.

**Results:** 65% patients were TB symptomatic with cough, fever, breathlessness and loss of weight being significant clinical features. Chest X-rays finding were infiltration and air space consolidation. Cavity was found in only 3% cases. 9 mycobacterium tuberculosis isolates were isolated. Mycobacteria were isolated from various samples viz. sputum (8 M. tuberculosis), blood (1 M. tuberculosis).

**Conclusion:** Mycobacterium tuberculosis is commonly isolated in AIDS patients.

**Keywords:** Mycobacterium tuberculosis; HIV and TB; AIDS

## Introduction:

Mycobacterial infections are the commonest opportunistic infections in Human Immunodeficiency Virus (HIV) or Acquired Immunodeficiency Syndrome (AIDS) patients. HIV infection is a potent risk factor for tuberculosis (TB). Not only does HIV increase the risk of reactivating latent Mycobacterium tuberculosis infection,[1] it also increases the risk of rapid TB progression soon after infection or re-infection with M. tuberculosis.[2-3]

In persons infected with M. tuberculosis only, the lifetime risk of developing TB disease ranges between 10% and 20%.[4-5] .In persons co-infected with M. tuberculosis and HIV, however, the annual risk can exceed 10%.[1] .Tuberculosis is one of the most common cause of morbidity and mortality in HIV-positive adults living in less-developed countries,[6] yet it is a preventable and treatable disease. M. tuberculosis causes majority of pulmonary and extrapulmonary infections and the risk is largely increased if the CD4 count falls below 300cell/cumm.[7] in some cases disseminated infections are also found.[8, 9]

Isolation of mycobacteria from clinical specimens other than blood is performed routinely in many of the laboratories these days. Blood samples which need special treatment and media are not routinely cultured [10,11] Likewise, in some of the TB patients with advanced HIV disease blood may be the only sample yielding M. tuberculosis.[12] Considering above, the present study was planned with following Aim and Objectives:

1) To isolate & identify Mycobacterium tuberculosis from various clinical samples collected from AIDS patients.
2) To perform antibiotic susceptibility testing for isolates using proportion method on LJ.

## Material and Method:

Attempts were made to collect the samples from the enrolled patients- 1.For mycobacterial culture-one blood sample, three sputum samples and any other extra pulmonary sample if available like of various tissues, fluids and aspirates. For CD4 counts- Blood sample - CD4 count testing was performed on fully automated two laser BD FACS Calibur Flow-cytometer USA using BD 4 color CD45/CD3/CD4/CD8 reagents. .For complete blood count- Blood was tested using Coulter Counter, X-ray chest - Performed on all patients recruited in the study.

### *Mycobacterial Culture:*

Blood samples were collected from all the subjects included in this study. Sputum and other



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pulmonary and extra pulmonary samples were also collected depending upon symptoms of the subjects, Collection and processing of blood samples*:* BACTEC™ 13A medium was used for isolation of mycobacteria from blood. 5 ml venous blood was inoculated directly into a 13A vial after swabbing the specimen with alcohol. 0.5 ml were tested every 2-3 days for first 2 weeks and weekly thereafter for a total period of 8 weeks. A growth index (GI) reading of 20 or more was considered positive for mycobacterial growth. Vials were reported negative if GI did not rise till the end of 8 weeks. Once the vial read positive, 0.5ml of the contents were aspirated using sterile 1ml syringe and 26G needle. The material obtained was stained by Ziehl-Neelsen (ZN) and Gram's staining techniques. If Acid Fast Bacilli (AFB) were observed on ZN staining, more material was aspirated from the vial and sub cultured onto 2 slopes of Lowenstein Jensen medium (LJ). One additional subculture was made into a fresh BACTEC 12 B vial to perform NAP test. If in a positive vial no AFB were seen on ZN staining, Gram stained smear was examined for the Gram reaction and the content were also sub cultured on blood agar and chocolate agar to isolate the organisms other than Mycobacterium .0.4ml of PANTA plus (Becton and Dickinson), was added to the vial immediately after subculture. There after the incubation and taking BACTEC reading was continued.

***Incubation of subcultures:***

The LJ subcultures were incubated at 37 $^{O}$C and read daily for 1$^{st}$ week and weekly thereafter for a total of 12 weeks. The growth was used for identification and to stock the strains.

***NAP test:***

When the subculture in 12 B vials reached a GI above 50, the BACTEC NAP test was performed following the recommended procedure. This differentiated MTB from NTM. Continuous growth in the presence of NAP (p-nitro-alpha-acetyl amino-beta hydroxypropiophenone) indicated NTM while inhibition of growth suggested M. tuberculosis. The NAP test could not be performed directly from the 13A vials because of the presence of blood in the medium.

***Processing Sputum samples:***

Smears were prepared and stained with Zeil Neelsen method .Smear was graded as per RNTCP. Sputum samples were decontaminated by Modified Petroff's method. Culture was done on 2 slopes of LJ medium and incubated at 37$^{0}$C and checked for growth daily for one week and then twice a week for 12 weeks after which they were discarded if no growth was seen .(Tuberculosis bacteriology, Collin, Grange and Yates, 2$^{nd}$ edition)

therefore from one subject multiple specimen were obtained depending upon the clinical features of enrichment (bovine serum albumin, 15.0 % w/v) was added to it. Inoculated vials were incubated at 37 $^{0}$ $\pm$ 1 $^{0}$C without shaking and tested next day onwards on BACTEC 460 TB instrument. The vials

The colour and colony characteristic of growth on LJ medium were recorded. The isolates were further subjected to the following tests in order to separate out the Mycobacterium tuberculosis from Non-Tuberculous Mycobacteria (NTM). All the tests performed for differentiation and identification were as per TRC manual 1987, Tuberculosis Bacteriology- Organization and Practice (Collins, Grange and Yates 1997), Practical Manual of Clinical Microbiology (Murray, 7$^{th}$ edition, 1999), Molecular Mycobacteriology manual and CDC manual 1975.

Phenotypic tests were performed and on the basis of which these mycobacteria were broadly classified in to M. tuberculosis and non-tuberculous mycobacteria. Niacin Test, Thermostable Catalase Test, semiquantitative Catalase test, nitrate reduction test

M. tuberculosis isolates were tested for drug susceptibility by proportion method on LJ. Drug sensitivity was performed for streptomycin, INH, rifampicin and ethambutol using H37Rv as control strain.

| Samples | Blood | Sputum | Stool | Others |
|---|---|---|---|---|
| To be collected | 94 | 162 | 188 | 21* |
| Collected | 94 | 144 | 148 | 14** |
| % collection | 100% | 88.89% | 78.72% | 66.67% |
| No. of isolates of M. tuberculosis | 1 | 8 | 0 | 0 |

**Table 1 : Number of various samples collected from AIDS patients**

* pleural fluid -5, LN aspirate -9, peritoneal fluid – 5, CSF -2

** pleural fluid -5, LN aspirate -2, peritoneal fluid.

## Results:

Considering the results of CD4 counts and clinical criteria, a total of 94 patients from 196 patients were diagnosed as suffering from AIDS as per CDC's revised classification system for HIV infections and AIDS[13] and were then investigated further. The patients (n=29) who had previous history of TB but not TB symptomatic at the time of study were excluded. The age of recruited patients ranged between 15 to 62 years with 74.47 % being male. Majority (91 %) of the male patients belonged to 25-54 years age group while females were restricted to younger age groups of 15-44 years (96 %) out of which 21 % were in the age group of 15-24 years, in contrast male in this groups were 1.4 % only. Among the males, 47 (67 %) out of the total of 70 and among females, 15 (63%) out of 24 were clinically diagnosed as tuberculous along with AIDS. 36 patients had symptoms of pulmonary TB, while nine had pulmonary TB along with pleural effusion (n=2) and lymphadenopathy (n=7).

| Findings | Number of samples |
|---|---|
| Smear + Culture + | 3 (5.56) |
| Smear + Culture - | 4 (7.40) |
| Smear- Culture+ | 5 (9.26) |
| Smear- Culture - | 42 (77.78) |
| Total | 54 |

**Table 2.: Correlation of smear and culture results of sputum samples**
Note: The figures in parentheses are percentages.

Seventeen patients had clinical features and or ultrasonographic evidence of extrapulmonary TB. Seven had lymphadenopathy. Five patients were diagnosed as having abdominal tuberculosis; three had pleural effusion while in two cases tuberculous meningitis was diagnosed.

Clinical samples were collected from 94 recruited patients. As per the protocol samples were to be collected for mycobacterial cultures (Table1).The sputum samples were also collected and processed for those patients who had cough of less than 2 weeks duration.

The results of phenotypic test are similar for all the nine isolates .They were niacin test positive ,heat stable Catalase test negative, Semiquntitative catalase test weakly positive nitrate reduction test positive. Amongst these 9 isolates, 8 were from sputum samples, 1 was from blood samples and no isolate was recovered from stool samples. In addition one CSF and four sputum samples showed acid fast bacilli on smear preparations which could not be isolated on culture. All these patients were on anti -TB therapy. In 19 (20%) AIDS patients out of 94, AFB were either seen on smear or grown on culture. Clinically 62 out of 94 (66%) patients had the diagnosis of TB with HIV infection. When smear positivity was taken into account the positivity of mycobacteria found to be 31%

Eight isolates from sputum were identified as M. tuberculosis. In the present study, AFB detection in sputum samples among 54 AIDS patients was 15.55% on microscopy alone and 17.77% on culture alone, while together the detection was 26.6% (Table2). Many of patients in the present study were terminally ill, TB patients who were unable to bring out good quality sputum for examination. After completion of phenotypic tests, the results were compared with those obtained by confirmatory tests. With respect to Mycobacterium tuberculosis, the correlation between phenotypic and confirmatory tests was 100%. The sensitivity of isolates of 9 M. tuberculosis isolates by proportion method, 3 were sensitive to all four first line drugs. Three other isolates were resistant only to Streptomycin and sensitive to other drugs. There were three isolates which were resistant to Rifampicin and Ethambutol, while they were sensitive to streptomycin and INH. No strain was found to be resistant to both INH and Rifampicin, thus no MDR TB was found in AIDS patients in this study.

In this study, chest x-ray findings showed that out of 62 clinically TB patients 45 (72.58 %) had positive x-ray findings. Infiltration and air space consolidation was observed in 34 cases (55%).

## Discussion:

As HIV-TB coinfection is high, all the HIV/AIDS patients are to be investigated for TB and if diagnosed are put on anti TB drugs before receiving ART. For patients who have not received antiretroviral therapy, the simultaneous initiation of treatment of both conditions has been associated with a high rate of side effects and paradoxical reactions.

Culture has advantages of identification of Mycobacteria as well as for their drug susceptibility testing. However, due to lack of infrastructure in the public health sector these techniques are not available at many places. In addition to smear examination, culture is also put for clinical samples by conventional LJ medium as well as using rapid BACTEC 460TB system. Speciation is

performed using phenotypic techniques. Drug susceptibility is put using proportion method on LJ.

The present study was undertaken to study the profile of these patients with respect to M. tuberculosis infection and the CD4 counts. The patients with CD4 counts <200 with or without TB and with counts >200 cells/cu mm with active TB were analyzed further. A total of 94 AIDS subjects were included in the study and amongst these 62(66%) were clinically suspected to be suffering from tuberculosis, 36 (58.06%) had only pulmonary disease, 17 (27.42%) had only extrapulmonary disease while 9 (14.52%) had both pulmonary as well as extrapulmonary TB . This distribution of disease pattern in a study from Mumbai among 176 TB patients was 115 (65%) with pulmonary, 28% extrapulmonary while 7% had both pulmonary and extrapulmonary disease.[14]The same figures reported in a study from Vellore were pulmonary 31.26%, extrapulmonary 21.87% and both 46.87%. [15] The dual site of infection in Vellore study was very high.

A total of 9 mycobacterial isolates were obtained from different clinical samples of 9 patients. Amongst these 9 isolates, 8 were from sputum samples, 1 was from blood samples. In addition one CSF and four sputum samples showed acid fast bacilli on smear preparations which could not be isolated on culture. In the present study AFB detection in sputum samples among 54 AIDS patients with was 15.55% on microscopy alone and 17.77% on culture alone, while together the detection was 26.6%. Many of patients in the present study were terminally ill TB patients who were unable to bring out good quality sputum for examination. In a study from New Delhi, AFB smear positivity was found to be 21.4%. In that study infiltrative lesions were found in 61.9% patients.[16]

Blood sample of one patient was also positive for Mycobacterium tuberculosis. Thus mycobacteremia was present in 6.4% AIDS patients .The mycobacterial isolates from blood was from TB symptomatic AIDS patients. Mycobacteremia suggests disseminated disease which was commonly observed in developed countries before advent of HAART.[17] In India, M. tuberculosis has been isolated from blood of AIDS patients in few studies carried out in Mumbai and Chennai.[8,9,18] In a study published from CMC Vellore blood cultures were done for isolation of M. tuberculosis in AIDS patients considering the fact that blood may be the only sample that might yield mycobacteria as mycobacteria containing samples are readily available for culture in such a patient. 93 consecutive blood samples were cultured using lysis centrifugation technique and in 4 cases blood was the only sample that yielded M. tuberculosis in addition to 11 other cases with mycobacteremia where the isolates were also available from other clinical samples.[8] Thus blood is a very useful sample for establishing the diagnosis of tuberculosis in AIDS patients.

The phenotypic identification in our laboratory, speciated the 9 isolates as M. tuberculosis. Thus, the correlation of phenotypic and genotypic method for M. tuberculosis which was the commonest isolates in HIV/AIDS patients was 100%.

Drug susceptibility of M. tuberculosis isolates showed that no MDR TB bacilli were isolated. Other studies from India have also shown that not many MDR TB strains are isolated from HIV/AIDS patients. In an extensive study carried out in AFMC Pune in HIV-TB coinfected patients, overall drug resistance was found to be 13.78% in HIV negative patients while it was 7.2% in AIDS cases. Similarly MDR TB was found in 8.9% HIV negative patients while it was 4.4% in AIDS patients[19]This is an important observation as contrary to the belief it clearly indicates that M. tuberculosis isolates from HIV/AIDS patients should be considered as sensitive unless proved otherwise.

The presentation of TB in early HIV infection, when the CD4 counts are more than 350cells/cumm, is like that of post primary TB; While , TB in advanced HIV disease or AIDS cases is like primary infection.

Patients who had M. tuberculosis isolated from their clinical samples were all young, with low body weight and had fever, cough, loss and weight and appetite. Night sweats were present in 3 cases. Fever along with low CD4 counts was thus a very important clinical parameter suggesting pulmonary TB and mycobacteremia.

## Conclusions:

Present study demonstrated the need for identification of tubercular infection when the CD4 counts <200 as they are important pathgenes causing disseminated infection in AIDS patients. Diagnosis of TB is based on sputum smear examination as per RNTCP and this examination has its own shortcomings even in HIV negative patients. In HIV/AIDS cases the sensitivity of sputum smear examination falls significantly as in the later stage of HIV disease clinical presentation of TB is the primary complex, chest X-ray findings are of infiltrative lesions without cavity and sputum smear examination is often negative.[20] Smear may also have low specificity as NTM will also appear as acid fast bacilli like M. tuberculosis.

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