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Characterization of Mycobacteriain Hiv/Aids Patients

KEYWORDS: *Mycobacterium tuberculosis*, NTM, AIDS, MPT64

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ABSTRACT *Background: Mycobacterium tuberculosis (MTB) is the most common life threatening opportunistic pathogen infecting HIV/AIDS Patients in developing countries. Recent studies have shown that incidence of non tuberculous mycobacteria (NTM) is also on the rise. Tuberculosis (TB) and HIV co-infection has put a huge pressure on health care facilities as there are many challenges in its diagnosis and treatment. Differentiating NTM from MTB is of utmost value as treatment strategies are totally different in these cases.*

Methods:In this prospective study of one hundred and six HIV seropositive individuals presenting with symptoms of pulmonary or extra-pulmonary TB, appropriate samples were collected and processed. The organisms were identified by microscopy(Ziehl-Neelsen staining)for acid fast bacilli (AFB), conventional culture [(Lowenstein-Jensen (LJ. media)] and automated culture(BACTEC MGIT 320) methods. MTB and NTM were differentiated by conventional methods and immunochromatographyassays using MPT64 antigen.

Results: Twenty two Mycobacteria were isolated from one hundred and six samples. Ten cases were positive on ZN staining, twelve on LJ where as nineteen were positive on MGIT. Out of 22 isolates 19 were M. tuberculosis and 3 were NTM (M.aviumcomplex 2 andM. fortuitum 1).

Conclusions: MGIT is better thanLJ medium & ZN stainingto detect Mycobacteria.MPT64 appears reliable with excellent sensitivityand specificityto differentiate between MTB and NTM.

INTRODUCTION

Human immunodeficiency virus (HIV)/ Acquired immunodeficiency disease syndrome (AIDS) and tuberculosis (TB) are the leading cause of death from infectious diseases worldwide[1]. While HIV / AIDS and TB can individually be the major causes for concern as stand-alone public health threats, the combination of the two has proven to have a far greater impact on the epidemiologic progression[2].

The interaction between HIV and TB in persons co-infected with them is bidirectional and synergistic. The course of HIV infection is accelerated subsequent to the development of TB and the inverse relationship between HIV viraemia and CD4+ count gets shifted to the right. The depletion of $CD4^+$ T cells, which is a main feature of AIDS, is certainly an important contributor to the increased risk of reactivation of latent TB and susceptibility to new *M. Tuberculosis* infection.

In developing countries TB is the most common life threatening opportunistic infection in patients with HIV/AIDS with about 25 to 65 per cent patients suffering from pulmonary or extrapulmonary tuberculosis[3,4].Unlike other opportunistic infections which occur at CD4+ counts below 200/mm, active TB can occur throughout the course of HIV disease. Clinical presentation of TB in HIV infected individuals depends on the level of immunosuppression resulting from HIV infection. In patients with relatively intact immune function (CD4+ count > 200/mm^3), pulmonary TB (PTB) is more frequently seen than extrapulmonary TB (EPTB).

Diagnosis of TB in HIV-infected patients is often difficult due to frequently negative sputum smears and higher prevalence of EPTB especially at inaccessible sites. So automated culture systems have been developed with great potential to detect the presence of MTB & NTM.

A large number of NTM are frequently found in the environment and can easily colonize in HIV/AIDS patients and cause disease, particularly in the advanced AIDS stage. Among the NTM, the most common opportunistic pathogen isolated is the *Mycobacterium avium* complex (MAC) which is associated with disseminated disease[5].

M. kansasii is the second most common NTM to affect patients with HIV/AIDS. Other NTM that are less frequently encountered include *M. fortuitum, M. chelonae, M.simiae*, M. *scrofulaceum, M. xenopi, M. genavense, M. haemophilum M. celatum, M. conspicuum, M.malmoense*. MPT 64 rapid antigen detection kit based on the principle of lateral flow immunochromatography has been developed to differentiate between MTB and NTM so as to put the patient on appropriate therapy.

METHODS

This prospective study was conducted over a period of one and a half year (1st January 2013 to 30th June 2014) in the Department of Microbiology, Government Medical College,Amritsar. A total of 106 HIV seropositive cases, clinically or radiologically suspected of pulmonary or extrapulmonary tuberculosis and attending Chest and TB Hospital/ART centre/Various OPDs/IPDs of Guru Nanak Dev Hospital, Amritsar were studied.

Appropriate clinical samples were collected from the patients depending upon signs and symptoms. Maximum number of samples collected were of sputum (52) followed by stool (20), pleural fluid (9), lymph node aspirates (9), endometrial biopsy (5), CSF (4), urine (4) and BAL (3). Samples were collected in sterile, wide mouth, screw capped

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containers aseptically and transported to the laboratory without any delay.

Direct ZN smear for acid fast bacilli was prepared. The NALC-NaOH procedure for digestion and decontamination was done according to standard protocol followed by preparation of concentrated ZN smear as well as inoculation on solid LJ media (conventional) and liquid media system i.e. MGIT (automated)[6]. MTB and NTM were differentiated by rapid immunochromatographic assays i.e. MPT64 Ag kit [7] and culture characters on LJ media such as growth rate, incubation at different temperatures, colony morphology, pigmentation, photoreactivity, sensitivity to p-nitrobenzoic acid (PNB) and a battery of biochemical tests [8]. Statistical analysis was done using SPSS software.

RESULTS

Out of 106 clinically suspected cases of tuberculosis, 22 isolates of *Mycobacteria* were obtained. 10 cases were positive on ZN staining for acid fast bacilli, 12 cases by conventional LJ culture method & 19 cases by automated MGIT culture method. Mean time for mycobacterial growth on LJ culture & MGIT was 31.92 days with a standard deviation of 10.54days and 13.42 days with a standard deviation of 3.79 days respectively.

Out of 22 isolates, 10 were positive only on MGIT culture, 9 were positive on both LJ medium culture and MGIT culture and 3 were positive only on LJ medium culture (Table 1).

Out of 10 smear positive cases on ZN staining for AFB, 90.00 % were positive on MGIT culture where as 80.00% were positive on LJ medium culture. Out of 12 smear negative cases on ZN staining for AFB, 83.33 % were positive on MGIT culture where as 33.33% were positive on LJ medium culture. In smear negative cases, there is considerable increase (50%) in positive cultures on MGIT as compared to LJ medium.

Table 1: COMPARISON OF LJ MEDIUM CULTURE AGAINST MGIT CULTURE IN SMEAR POSITIVITE AND SMEAR NEGATIVE POSITIVE CULTURES

Smear(ZN staining) Results	No. of Positive Cultures (% recovery)	
	LJ medium culture	MGIT culture
Smear positive (10)	08(80.00%)	09(90.00%)
Smear negative (12)	04(33.33%)	10(83.33%)
Total (22)	12	19

Out of 10 smear positive cases on ZN staining for AFB, 90.00 % were positive on MGIT culture where as 80.00% were positive on LJ medium culture. Out of 12 smear negative cases on ZN staining for AFB, 83.33 % were positive on MGIT culture where as 33.33% were positive on LJ medium culture. In smear negative cases, there is considerable increase (50%) in positive cultures on MGIT as compared to LJ medium.

Out of 22 isolates, 19 isolates (86.36%) were MPT 64 antigen positive i.e. *M tuberculosis complex* (MTBC) and only 3 isolates (13.63%) were MPT 64 antigen negative (NTM). On LJ media same results were obtained. Among NTM, two isolates of MAC and one isolate of *M. fortuitum* was obtained.

Out of 19 HIV seropositive cases infected with MTBC ,15(78.94%) cases were having CD4 count in the range 101-200 cells/mm^3, 3 (15.78%) were having CD4 count in the range 201-300 cells/mm^3 where as 1(05.26%) was having CD4 count <100 cells/mm^3.

Pulmonary TB was found in majority (72.72%) of the HIV-TB coinfected cases as compared to extrapulmonary TB (27.27%). Pleural effusion (09.09%) accounted for majority of extrapulmonary TB cases followed by lymph node TB (04.54%), tuberculous meningitis (04.54%) and gastrointestinal (GIT) TB (04.54%).

DISCUSSION

In Indian scenario, mycobacterial infections are the commonest opportunistic infections in HIV patients. There should always be a high index of suspicion for diagnosis of TB in HIV as clinical features are atypical due to immunosuppression.

Conventional method such as ZN staining is rapid, easy and cheap method but diagnosis of TB is still dependent on culture because it is more sensitive than ZN staining as it can detect the mycobacteria even in paucibacilliary specimens.

In the present study, sensitivity, specificity, positive predictive value, negative predictive value and accuracy of ZN staining for AFB against culture on LJ medium was found to be 66.67%, 97.87%, 80%, 95.83%, 94.34% respectively. This may be explained on the fact that as few as 10 to 100 bacilli per ml can be detected by culture while at least 10,000 acid fast bacilli should be present/ml of smears for them to be readily demonstrable in direct smears for AFB. Similar results were obtained in a study conducted by Kaur KP et al[9]. In our study, recovery rate of mycobacteria on LJ was 12/22 (54.54%) against 10/22 (45.45%) on ZN staining which was not found to be statistically significant (p value=0.546). Narang P et al also observed 52.3 % and 38.9% recovery rate of mycobacteria on LJ and ZN staining respectively. In the present study, recovery rate of mycobacteria on MGIT was 19/22 (86.36%) which was found to be statistically significant as compared to ZN staining (p value=0.004). Kaur KP et al also observed similar results in her study[9].

Recovery rate of mycobacterium on LJ medium and MGIT in the present study was 54.54% and 86.36% respectively. Increased sensitivity on MGIT as compared to LJ may be explained by the more inoculum size, better quality of the media and an automated system. In our study sensitivity, specificity, positive predictive value & negative predictive value of culture on LJ medium against culture on MGIT for TB was 47.37%, 96.55%, 75.00%, 89.36% and 87.74% respectively. These results were in concordance with results of a study by Barreto LBPF et al who also found sensitivity, specificity, positive predictive value and negative predictive values of culture on LJ medium against MGIT as 88.6%, 92.4%, 83.8%, and 94.8%, respectively[10]. In our study, mean time for growth detection on LJ medium was 31.92 days with a standard deviation of 10.54 days and on MGIT, it was 13.42 days with a standard deviation of 3.79 days. This may be explained as MGIT is more sensitive than LJ so it detects mycobacterial growth more rapidly.

In smear negative cases, there was considerable increase of 50.00% in positive cultures in MGIT as compared to LJ, which otherwise would have been missed on LJ. The more inoculum size, the quality of the media, and a fully automated and continuously monitored system adds to increased sensitivity in MGIT. Kaur KP et al also observed in her study that in smear negative specimens,100% were

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positive on MGIT where as 33.3% were positive on LJ[9].

The liquid media system i.e. BACTEC MGIT 960 system is better than solid Lowenstein-Jensen media to detect growth of mycobacteria inspite of the problems such as contamination and high cost because it is easy to use, dependable, high-capacity, compact, fully automated, continuous monitoring system that expedites the growth and recovery rate of MAC and NTM where as LJ culture requires long incubation period and is labour intensive. However the combined use of LJ and MGIT could be justified for maximum recovery of isolates as neither MGIT nor LJ is able to recover all the mycobacterial isolates.

In our study, out of 22 isolates, 19 isolates (86.36%) were MPT 64 antigen positive i.e. *Mycobacterium tuberculosis complex* and only 3 isolates (13.63%) were MPT 64 antigen negative which were reported as NTM. As MPT 64 antigen kit detected all the 22 isolates correctly in concordance with biochemical tests to characterize mycobacteria so it was found to be 100% sensitive and specific. Study by Toihir AH et al also concluded that the MPT64 antigen for TB has excellent sensitivity (100%), and specificity (100%)[11]. The sensitivity, specificity, PPV and NPV of MPT-64 antigen for TB was 99.19%, 100 %, 100% and 97.3% respectively as studied by Swapna[12].

Nontuberculous mycobacteria infection is one of the leading cause of opportunistic infections in patients with advanced acquired immunodeficiency syndrome i.e. with CD4 count less than 50/cu.mm[13]. In the present study, 2(66.66%) cases infected with NTM were having CD4 count<100 cells/mm^3. A study by Khatter S et al who observed 37.50% cases infected with NTM were having CD4 count<100 cells/cubic mm[14]. In our study, out of 19 HIV seropositive cases infected with MTB , 16(84.21%) were having CD4 count<200. Findings similar to our study were also observed by Gautam H et al, who observed 100% of the cases infected with MTB were having CD4 count less than 200[15].

The high proportion of NTM in HIV seropositive individuals underscore the need for rapid speciation tests as HIV-NTM coinfection presents a novel public health challenge, which needs to be considered when planning for prevention and treatment of these patients with standard TB regimens since their response to these regimens are known to vary from *M. tuberculosis*. Recently kits based on detection of excretory antigen i.e. MPT64 have emerged as a promising tool to differentiate between MTB and NTM and appear better than phenotypic characterization for the management of TB. Liquid culture and molecular species identification tests have seldom been used in resource constrained settings, largely because of cost however these techniques have the potential to reduce mortality by facilitating rapid diagnosis of smear-negative TB since HIV-infected patients are more likely to have smear-negative TB.

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