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Evaluation of various techniques among clinically suspected patients of pulmonary tuberculosis with and without the presence of HIV infection

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Abstract: *Tuberculosis (TB) remains a major global health problem in India and globally. The present study was conducted to evaluate the different diagnostic techniques in suspected cases of pulmonary tuberculosis with or without the presence of HIV (Human Immunodeficiency Virus) infection. A total of 75 clinically suspected fresh cases of pulmonary TB were asked for sputum samples for acid-fast bacilli (AFB) staining and culture on Lowenstein-Jensen (LJ) media according to Revised National Tuberculosis Control Program guidelines. A commercially available rapid test kit was also used for detection of TB antigen in sputum sample. One 5 mL blood sample each was taken for HIV testing according to National Aids Control Organisation guidelines. A total of 9 (12%) patients out of 75, were found to be HIV-positive. Among the HIV co-infected patients, there was only one patient who was both smear and culture positive rest were negative. On the other hand, among the 66 HIV-negative TB patients, 25 (37.88%) were positive on both smear and culture. None of the smear negative and culture negative samples was found to be positive for the presence of TB antigen. The overall antigen positivity rate in the entire study was 16% (12/75). The current study concluded that LJ media is highly efficient medium for recovery and diagnosis of Mycobacterium tuberculosis.*

Keywords: *HIV; AFB; LJ media; pulmonary tuberculosis; TB antigen*

I.Introduction

In 2012, an estimated 8.6 million people developed tuberculosis (TB) and 1.3 million died from the disease (including 320 000 deaths among HIV-positive people) [1]. The co-infection of Human Immunodeficiency Virus (HIV) and Mycobacterium tuberculosis (MTB) is associated with major diagnostic problems since HIV infection often leads to extra pulmonary and smear negative pulmonary tuberculosis. X-rays abnormalities, which are not specific for TB in HIV-negative patients, are even more non-specific, with only minor abnormalities that do not look like classical tuberculosis, in the HIV-infected. In addition, patients infected with HIV have frequent illnesses with pulmonary involvement caused by agents other than MTB [2].

Since the behavior pattern of TB/HIV association differs from region to region, different diagnostic approaches are required region to region. In view of this and the absence of any data on this aspect in this region, the present study was conducted to evaluate the different diagnostic techniques among suspected cases of pulmonary tuberculosis with or without the presence of HIV infection.

II.Material And Methods

This prospective study was carried out in the Department of Microbiology, over a period of twelve months, at a tertiary care hospital that also operates as a center for the Revised National Tuberculosis Control Program (RNTCP). The institutional Ethical Committee approved this study. A total of 75 clinically suspected fresh cases of pulmonary TB were asked for sputum samples for acid-fast bacilli (AFB) staining and culture according to RNTCP guidelines [3]. A commercially available rapid test kit was also used for detection of MTB antigen in sputum sample. One 5 mL blood sample each was taken for HIV testing according to NACO (National Aids Control Organisation) guidelines [4].

All clinical sputum specimens were first processed for Ziehl-Neelsen acid-fast staining and then for culture. The culture was done on Lowenstein-Jensen (LJ) media following homogenization and decontamination of sputum sample by modified Petroff's method. The processed specimen was incubated at 37ºC for a maximum of eight weeks and read once weekly. The identification process of MTB comprised the phenotypic identification of cultures of acid-fast bacilli grown on solid medium based on the combination of observation of colony morphology (Fig. 1), inability to grow on a culture medium containing PNB (p-nitrobenzoate) and results of biochemical tests specific for thermolabile catalase, nitrate reductase and niacin accumulation test [3]. Reference strain H37Rv was used for quality control.

Antigen detection was done in sputum samples, using commercially available rapid test kit called,

Mycobacterium tuberculosis Antigen Rapid Test (strip), manufactured by Jei Daniel (JD) Biotech Corp. Shandong, China. It is a chromatographic lateral flow immunoassay, in which 300 µl of sputum sample, 40 µl buffer and 960 µl distilled water were taken in an ependorf tube. After vortexing for one minute, antigen detection strip was dipped in the tube for 60 minutes and observations were made after the interval of 15 minutes. A rose pink line in the test zone along with control zone were taken as indicative of the presence of TB antigen in the sample. Appearance of control line in absence of a test line was taken as a negative test (Fig. 2).

The serum samples were analyzed for the presence of HIV antibodies as per NACO guidelines [4]. HIV testing was carried out according to the following algorithm using three test kits, viz. HIV COMB (J.Mitra & Co.), HIV TRIDOT (Biomed industry) and Microlisa-HIV ELISA (J.Mitra & Co.).

Fig. 1; growth of Mycobacterium tuberculosis on LJ egg medium

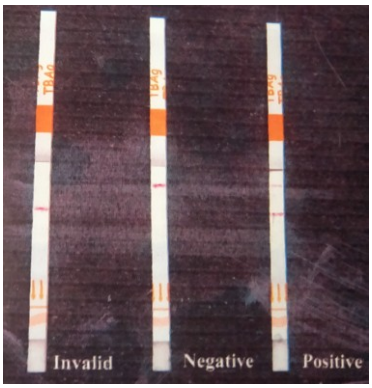

Fig. 2; photograph showing different results in TB antigen detection strips

III.Results

In the present study, a total of 9 (12%) patients out of 75, were found to be HIV-positive. It was observed that among the HIV co-infected patients, there was only one patient who was both smear and culture positive rest were negative. On the other hand, among the 66 HIV-negative TB patients, 25 (37.88%) were positive on both smear and culture. Two (3.03%) patients were positive on culture but negative on smear examination. A total of 39 (59.09%) patients were negative on both smear and culture examination. None of the smear negative and culture negative samples was found to be positive for the presence of TB antigen. The positivity rate was 46.2% (12/26) and 42.9% (12/28) among the smear and culture positive patients respectively (Table 1).

However, comparing the diagnostic sensitivity of smear microscopy (26/75 i.e. 34.67%) in the present study with that of TB antigen detection test (12/75 i.e. 16%), it was found that the former was significantly more sensitive compared to the later (p=0.005). Similarly the diagnostic sensitivity of LJ culture (28/75 i.e. 37.33%) was significantly higher compared to TB antigen detection test (p=0.002) (Table 1).

Table 1; comparison of smear, culture and antigen positivity rate between HIV-positive and HIV-negative patients

(n=75)	HIV-positive (n=9)	HIV-negative (n=66)
Smear microscopy	1 (11.11%)	25 (37.88%)
LJ culture	1 (11.11%)	27 (40.9%)
TB antigen	1 (11.11%)	11 (16.67%)

IV.Discussion

Tuberculosis 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) [1]. The co-infection of HIV and TB is also associated with major diagnostic problems. The present study showed HIV-positivity rate of 12% among the recruited patients. A study conducted in New Delhi on 555 patients with TB demonstrated an HIV seropositivity of 9.4%, vs an overall seropositivity in this same hospital of 0.4% from 1994-1999 [5].

In this study, sputum smear positivity for AFB was significantly lower in HIV-positive cases than in their HIV-negative counterparts. This is an agreement with a number of studies reported from several countries across the globe, all of which reported that HIV-positive individuals are less likely to be smear positive than HIV-negative individuals [6,7]. The frequency of smear positive individuals has been demonstrated to correlate with the immune status. A relatively better immune status is associated with smear-positive disease, whereas increased impairment of immunity carries higher chances of sputum smear negativity [8]. Liberato et al. have also reported that over 50% of co-infected patients in Brazil were negative for acid-fast bacilli in the sputum [9].

The frequency of culture positivity of sputum samples on LJ media among HIV co-infected patients compared to their HIV-negative counterparts was significantly low. Though mycobacterial culture is recommended for diagnostic purposes in smear negative pulmonary tuberculosis its yield is comparatively lower in HIV-positive TB patients due to lower bacterial concentration in their sputum samples. A study from Brazil reported that patients who were HIV seropositive had a lower frequency of positive sputum culture for Mycobacterium tuberculosis in comparison with those who were HIV-negative [9].

In view of the diagnostic difficulties associated with TB/HIV co-infection, this study was undertaken to assess the performance of a TB antigen detection kit. While none of the smear negative samples showed a positive reaction in the test, 12 of the 26 smear positive patients demonstrated the presence of TB antigen in their sputum samples. The lone smear-positive HIV patient was also found to be positive for the detection of TB antigen in sputum sample. Kashyap et al. (2007) used an indirect ELISA for demonstrating Ag 85 complex in sera from TB patients using a monoclonal antibody against the complex that yielded the 82% sensitivity and 86% specificity [10]. In another study reported form Tanzania, Boehme et al. (2002) assessed the diagnostic potential of a direct antigen capture ELISA based on the detection of mycobacterial lipoarabinomannan antigen (LAM) in unprocessed urine samples that showed the 80.3% sensitivity and 99% specificity [11]. Kameshwaran et al. (1989) using a simple dot ELISA for the detection of MTB Antigen in sputum demonstrated the 61% positivity in smear positive samples and 39.6% positivity in smear negative samples [12]. The remarkable difference between the performances of the antigen detection kit assessed by us and the ones reported in the literature could be accounted for by the differences in the nature of the antigen detected in the respective kit. However, to the best of our knowledge, this is the first effort to explore the diagnostic potential of TB antigen detection in TB-HIV co-infected patients.

V.Conclusions

HIV co-infection of TB patients is a significant problem that presents unique treatment problems and poses a public health threat. This study found a high prevalence of HIV co-infection in adult TB patients. The overall antigen positivity rate in the entire study was 16%, thereby making it unsuitable for routine diagnostic use. Smear microscopy is limited due to low sensitivity in paucibacillary specimens. On the other hand, culture remains the gold standard for diagnosis of mycobacterial infections, although it is time-consuming process but we can rely on this method.

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