Diagnosing Sputum/smear-negative Pulmonary Tuberculosis in PLHIV Based on Culture a Study from MGM District Hospital

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ABSTRACT

Diagnosis of sputum/smear-negative pulmonary tuberculosis patients can be both challenging and time consuming with many patients being put on empirical anti-tubercular treatment. The study was conducted on100 suspected sputum smear negative pulmonary tuberculosis causes attending ICTC of MGM District Hospital Warangal. All the suspect's sputum was screened for AFB staining were shown negative. Their mode of clinical presentation, site of TB, CD4 cell count, dysentery in HIV, TB, studied. Tuberculosis (TB) was found in 15% of HIV infected patients those were showing smear negative for AFB. TB were significantly more common in HIV seropositive patients (p< 0.02) in whom the CD4 cell count was also low (less than 100/cmm).8% of dysentery was found among them. The result of this study showed the increased vulnerability of the HIV infected persons to TB infection as seen in other studies in India and abroad. Disseminated TB with lower CD4 cell count is also shown. Smear negative persons were more prone for tuberculosis infection which may become problem in diagnosis and treatment of tuberculosis.

Key words: HIV/AIDS, Smear negative, TB, CD4 Counts, diherial.

INTRODUCTION

India has 20% of the global burden of tuberculosis infections, with 1.9 million new cases each year, as well as a large pool (2.5 million) of HIV-infected individuals[1,2]. Out of 1.9 million registered for treatment of tuberculosis in India 28.7% of them were new smear negative cases[3]. Tuberculosis (TB) is a leading cause of HIV-related deaths worldwide. Approximately 30% of HIV-infected persons are estimated to have latent TB infection through out world[4]. The mortality associated with TB is considerably higher in HIV-infected than HIV-negative patients[5]. The HIV epidemic which is leading to increase in the frequency of smear-negative pulmonary tuberculosis, compared to the smear positive cases, it becomes a complication in diagnosis of HIV persons. HIV-infected patients are twice as likely to have sputum smear-negative, culture-positive pulmonary TB (PTB). The sputum smear has traditionally been used as the method for making an early diagnosis of PTB. Sputum smear examination for acid- fast bacilli (AFB) may detect up to 15-40% of pulmonary tuberculosis cases in laboratories and large portion may remain negative in spite of clinical profile and radiological lesions being consistent with diagnosis of pulmonary tuberculosis[6]. The reduction in sensitivity of this test in HIV-infected patients leads to diagnostic delay. In the TB screening process the Chest X-ray (CXR) is an indispensable tool in PLHIV, for whom sputum is often negative[7]. Chest X-ray shortens delays in diagnosis of TB and non-TB chest diseases common which are among people living with HIV. HIV TB Co-infected patients with signs and

symptoms may die before sputum or culture is available by using Digital Radiology, Chest images are instantly available and abnormalities consistent with TB can be detected on the spot. When patients showing with CD4 cell counts less than 200 cells/uL show less typical abnormalities in their Chest image, then it becomes complicated in identify the positive ness of TB. Sputum culture is a more sensitive method of diagnosing PTB in such cases, but will takes up to 8 weeks for the result to be available[8]. Early diagnosis of pulmonary tuberculosis prevents progression of disease, morbidity, spread of disease and permanent damage by fibrosis with normal individual to HIV positive individuals[9]. HIV as a syndrome associated with other bacterial infections that were mainly belong to the class of Mycobacterium and Enterobacteriaeceae members, it is obvious that HIV positive patients were more susceptible to mycobacterial and diarrheal infections[10]. CD4 and CD 8 counts vary in pure HIV positive patient and HIV patient with other co infections[11]. Observations from many parts of the world have shown higher incidence of TB among HIV infected individuals, ranging from 5 to 10 per year of observation[12]. The mortality associated with TB is considerably higher in HIV-infected than HIV-negative patients[10]. The present study aims to assess the role of culture in the diagnosis of sputum/smear-negative pulmonary tuberculosis.

MATERIAL AND METHODS

For the assessment of the process we have compiled nearly 100 HIV positive patients 'blood samples, from district hospital, Warangal and subjected to CD4 count and also recognition of the other bacterial infections in HIV patients. This method consists of series of steps mainly Sample collection and handling, CD4 cell counting in blood using Flow cytometry, Identification of other bacterial infections associated with HIV and confirmatory tests to determine the particular organism coupled with HIV infection.

Sample collection and Handling

For CD4 enumeration by flow cytometry, whole blood specimens was collected by venipuncture using evacuated blood collections tubes with K3-EDTA or K2-EDTA as the anticoagulant (13). All blood samples labeled at the time of specimen collection, and transported to the Flow cytometry chamber the temperature maintained is18°c to 22°c. Clotted specimens were not used for the study.

CD4 enumeration by Flow cytometer

For the CD4 cell enumeration BD FACS calibur cytometer were used, each patient sample labeled by BD trucount tube with sample identification number. 20µL of BD TRITEST CD3/CD4/CD45 reagent pipetted out in to the bottom of the tube (14). Using the reverse pipetting technique sample is pipetted out on to the side of the tube just above the retainer. 50µL of well mixed anti coagulated whole blood pipetted out in to the bottom of the tube. Tube and vortex capped and mixed gently, after this tube is incubated for 15 minutes in the dark room temperature 20°c-25°c. Later 450µL of 1× BD FACS lysing solution is added in the tube and mixed gently; the sample is incubated for 15 minutes at dark room temperature 20°c-25°c. Finally the sample is analyzed on the flow cytometer (15).

Identification of the other bacterial infections and Confirmatory examinations

Sputum and stool sample were collected. Sputum specimens were collected was about 5-10 ml which was processed on the same day as per guidelines[16]. The clinical sputum specimens submitted for the determination of possible mycobacterial infections were examined first for acid-fast bacilli. Smear made from the collected sputum specimen were stained by the Ziehl-Neelsen and examined by microscopy to detect the cases of mycobacterial infections, this method served as an adjunct to culture for determining the acid- fast characteristics of bacteria[17]. For the betterment of acid-fast staining of a smear and to reduce the viscosity of specimen, the specimen to be examined was initially treated with 5% of sodium hypochlorite with an equal volume of specimen (18). The concentration of bacilli by centrifugation was done by the Petroff"s method[19]. The homogenized sputum was cultured on, Lowenstein Jensens (LJ) media[20]. LJ media slopes were used for inoculation of sample and incubated as, first slope at 37 ºC, second slope wrapped in black paper at 37 ºC, third slope at 25 ºC and fourth slope at 44 ºC. Bacteria obtained after incubation on different slants

were subcultured and different parameters *viz.,* Rate of growth[21], colony morphology[22], temperature at which growth occurs[23], production of pigment in light and dark[24], Niacin test[25], Nitrate reduction test[26], Catalase test[27], Growth on MacConkey agar[28], Tellurite reduction test[29], Urease test[30]and Tween80 hydrolysis test[31] were performed on the isolates, which are 3-4 weeks old to identify the type of mycobacterium. In the same way diarrheal have been identified by culturing the stool sample on Nutrient agar media, confirmatory test have performed by biochemical and motility tests, reveals diarrheal infection along with HIV is caused by E. coli.

Results and Discussion

Of the 100 seropositive cases, 56 were males and 44 were females. There CD levels had shown a vared in proportions during there attempt to ICTCs in MGM Warangal, district hospital. Out of the 100 patients shown lower CD4+ counts below 200 rate is high in males, 9 HIV seropositive males were found and 5 cases of females were found in our study. The age range for seropositive patients was from 16-46 year. The highest number of HIV infection was found in the age group of 21 - 30 years 60. The seropositivity rate was highest among those who were unemployed is about 40. Tuberculosis was found in 20 patients in the seropositive group (age ranged from 21 - 36 years). HIV seropoistive patients shown a negative AFB smears. Out of 100 HIV Seropositive patients 15 where positive for the culture on L-J medium. Based on morphology, biochemical tests, and growth rates of the bacterium it was identified as *M.tuberulosis.* Of the 100 HIV positive, 8 patients shown diherial infections, when stool samples were subjected for the culture on MacConkey, Nutrient Agar medium, growth was found to be *E.coli*.

In Asia where HIV epidemic is at an early stage, surveillance data show that the rates of HIV infection had remained lower in-patients with TB compared to that seen in Africa. Studies form Uganda and Zambia have recorded HIV rates of 50-70% among TB patients[32]. In Tanzania, the survey conducted during 1994-l 998 on 10,612 new smear positive TB patients revealed 40% HIV prevalence. HIV sero-prevalence rates among patients with extra-pulmonary TB are even higher extra

pulmonary TB has been reported in upto 70% of HIV related TB cases when the CD4 lymphocyte counts falls below 10025. Studies indicates that the HIV sero-positivity in TB patients show a wide variation ranging from 0.4% in a study in Delhi to 28.75% in a study conducted in Pune. Moreover, periodic studies from some centres indicate that the HIV prevalence is rapidly increasingamong TB patients[33]. In our experience, about 2/3rd of HIV infected individuals have a CD4 < 200 cells/mm3 when they present with TB. Hence, the earlier use of ART is likely to reduce mortality and morbidity in this group of patients[34].

In this study, TB was found in 55 % of the HIV positive patients and in 25% of the HIV negative patients which was significant statistically (p < 0.001). This is consistent with the findings in another study7, but higher than that of a Kolkata study with 27.7% only. Our study was based on the patients admitted in the hospital for evaluation of fever. This may explain the higher incidence of TB in those seronegative patients.

CONCLUSION

HIV has a world wide distribution and is already a huge problem of over stretching the fragile health infrastructure in most of the countries, like India, with the increasing tuberculosis cases load with HIV infections there will be greater demand to d diagnose and treat HIV TB cases. The diagnosis of TB in HIV patients is difficult for many reasons. Our study had fouced on the seropositive for HIV and when they were subjected for testing for oppurtunictic infections surpricingly to our knowleged we found smear negative for AFB staining TB were shown culture. Present study revealed evidence of CD4 levels in HIV-TB infections was low CD4 > 200 cells /mm. Out of 100 positive HIV causes there were 15 smear negative but culture positive cases for tuberculosis bacilli. 15 isolates were identified as Mycobacterium tuberculosis, 8 isolstes of *E.coli*, were also found in our study.

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