

Isolation and Characterization of Mycobacterial Species in Clinically Diagnosed Cases of Pulmonary Tuberculosis and Few Associated with HIV Infections

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Tuberculosis (TB) is presently recognized as one of the most common opportunistic infections seen in HIV seropositive patients, mostly presenting in the form of pulmonary and extrapulmonary infections. A total of 100 clinically diagnosed and radiologically evident cases suggestive of pulmonary tuberculosis were selected for study. Sputum sample of each patient was screened for Acid Fast Bacillus (AFB) by Ziehl Neelsen staining and cultured. On examination 70 smears were found positive for AFB and 30 smears were negative by concentration method. A total of 27 samples were found to be culture positive and 73 were culture negative, of these 26 stains were identified as *Mycobacterium tuberculosis*, one was identified as *M.avium complex* (MAC). Of the 100 patients 15 were seropositive for HIV-I antibodies. Out of 15 seropositive patients, 2 patients smear and culture were positive and identified as *M.tuberculosis*, one patient shown culture positive and smear negative and the other patients culture and smear were both negative for AFB.

Key words: Acid Fast Bacteria, HIV infection, *Mycobacterial* species, Pulmonary tuberculosis.

The organism responsible for tuberculosis was identified and diagnostic tests were developed more than 100 years ago, a tuberculosis vaccine has been in use for over 60 years, and chemotherapy for over 30 years. Despite of all these, developments tuberculosis still remains a major international health problem. Tuberculosis (TB) has been declared as a global health emergency by the World Health Organization (Patwardhan *et al.*, 1997). In individuals infected with *M. tuberculosis*, chance of progression to tuberculosis is more, if they are also infected with human immunodeficiency virus, tuberculosis may

accelerate the evolution of HIV infections to overt disease, i.e., AIDS is dually infected in individuals. The rise in TB incidence over the last two decades is due to tuberculosis deaths in HIV-infected patients. Centre for Disease Control and prevention in USA has included TB as an additional AIDS indicator disease (Vijayan *et al.*, 2002). TB infection occurs as opportunistic infections in HIV infections. Timely diagnosis and treatment of TB is important, not only for patients of HIV, but also for other respiratory infections. HIV multiplies the problem of TB during its course of HIV infection it also complicates. Majority of TB patients suffer from pulmonary lesions and the recommended tools of diagnosis for pulmonary TB are smear microscopy and chest radiography (Toman *et al.*, 1981). Culture of *mycobacteria* has been identified as the Gold standard (Sethi, *et al.*, 2005) for diagnosis of pulmonary TB disease. Culturing is

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more sensitive than sputum smear microscopy. Culturing is also useful in sputum smear negative cases where active tuberculosis is suspected. With the problem of tuberculosis rising in this area, we undertook this study in our region.

MATERIALS AND METHODS

The present investigation was done at Department of Microbiology, Kakatiya Medical College, Warangal, INDIA. The clinical examination of patients and collection of clinical samples were made at District Tuberculosis Center, Warangal, INDIA. Samples were collected from clinically and radiologically diagnosed patients of TB infection with different age and sex. The study included 100 cases of pulmonary TB irrespective of antitubercular treatment. Sputum sample were collected in a sterile 50-ml, screw- capped centrifuge tubes or sputum cups of disposable type (HiMedia, Mumbai, India). Sputum specimens of about 5-10 ml were collected and processed on the same day as per CDC guidelines (Patricia and Georgia, 1985). 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 (Herbert and Robert, 1985). 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 (Krawnsnow and Wayne, 1969). The concentration of bacilli by centrifugation was done by the Petroff's method (Monica Cheesbrough, 1991). The homogenized sputum was cultured on, Lowenstein Jensens (LJ) media (Krasnow and Kidd, 1965). LJ media slopes are used for inoculation of sample and incubated as, first slope at 37 °C, second slope wrapped in black paper at 37 °C, third slope at 25 °C and fourth slope at 44 °C. Bacteria obtained after incubation on different slants were subcultured and different parameters viz., Rate of growth (Herbert and Robert, 1985), colony morphology (Herbert and Robert, 1985), temperature at which growth occurs (Herbert

and Robert, 1985), production of pigment in light and dark (Minnikin, 1982; David, 1978), Niacin test (Kilburn, *et al.* 1968), Nitrate reduction test (Jenkins, 1985), Catalase test (Kubica, *et al.* 1960), Growth on MacConkey agar (Jones, 1964), Tellurite reduction test (Kilburn, *et al.* 1969), Urease test (Collins, *et al.* 1985) and Tween80 hydrolysis test (Vestal, *et al.* 1975; Kilburn, *et al.* 1973) were performed on the isolates, which are 3-4 weeks old to identify the type of mycobacterium.

All the reagents of analytical grade and media used in this study were purchased from HiMedia Laboratories, Mumbai, India. Difcon niacin strips were supplied by Remel, Lenexa, USA. 5ml of blood was collected with aseptic precautions in vacutanenir, Serum was used to detect antibodies to HIV-I and II by Tridot test method to confine the seropositiveness of HIV patients.

RESULTS AND DISCUSSION

Acid fast staining of the smear showed pink colored rod shaped bacilli. Out of 100 smears including 15 HIV seropositive, 70 (70%) were positive for acid fast staining, remaining 30 (30%) were negative. Smear positive and smear negative samples were for culture. Growth of acid fast bacteria was obtained at 37 °C in a CO₂ atmosphere. Growths of acid fast bacilli were seen in 27 samples and are designated as culture positive. Remaining 73 samples were designated as culture negative. Anuradha *et al.*, (2001) reported out of 392 randomized samples 5 were positive for culture. Of the 27 culture positive samples 21 were both smear positive and culture positive, and the other 6 are smear negative and culture positive. Of 15 HIV seropositive samples 7 were positive for acid fast staining and 3 were culture positive. Of the 225 patients, of which 6 were HIV positive (2.7%). Out of these 6 cases, one (16.7%) was smear and culture negative and the remaining 5 (83.3%) were smear and culture positive for AFB and identified as *M. tuberculosis* (Shivaraman *et al.*, 2001). Many sputum specimens shown smear and culture negative may be due to irregular release of mycobacteria from endobronchial foci may result in a variable pattern of positive and negative smears from the patients or by inactivation of tubercle bacilli by drugs or by excess toxic decontamination procedures.

All the sputum samples inoculated on the Lowenstein Jensen (LJ) medium, 26 isolates were showing characteristics of *M. tuberculosis*, which were recognized by their slow growth, rough surface with buff tint. One isolate showed characteristics *M. avium* complex, which was recognized by its smooth surface and thin, domed and eugonic in shape. Growth of these cultures was acquired after 4 to 6 weeks or longer incubation period. Herbert and Robert (1985) reported that mycobacterium spp. require longer incubation period. Cultured colonies were unable to produce pigmentation either in dark and light exposure of the colonies indicating that these are non photochromogenic which includes *M. tuberculosis* or *M. avium* (Minnikin, 1982; Herbert and Robert, 1985). Konemn *et al.*, (1997) studied *M. avium* complex is now the most frequent mycobacterium other than *M. tuberculosis* isolated in the untied states. Infection with *M. tuberculosis* complex has become almost common in patients with acquired immunodeficiency syndrome (AIDS).

26 cultured colonies were able to produce niacin abundantly indicating that these are *M. tuberculosis* and the one which didn't produce niacin is *M. avium* (Kilburn, *et al.*, 1968). 26 cultures produced pink to red coloration for nitrate reduction test which is a valuable for the identification of Mycobacterial spp. indicating *M. tuberculosis* and the one which didn't produce is *M. avium*. (Jenkins, 1985). All the cultures were positive for catalase test indicating non isoniazid resistant strains (Kubica *et al.*, 1960). Growth was not observed on MacConkey agar indicating the absence of *M. fortuitum* (Jones, 1964). Only one strain produced jet black precipitate for tellurite reduction test indicating *M. avium* strain (Kilburn *et al.*, 1969). 26 cultures were found to be positive for tween 80 hydrolysis test by its color change from amber to red. Non photochromogenic strains like *M. tuberculosis* responds to this test where as *M. avium*, *M. xenopi* and others are negative (Vestal *et al.*, 1975; Kilburn *et al.*, 1973). All the cultures doesn't hydrolyzed urea by urease test indicating that one culture which was not *M. tuberculosis* was confirmed to be *M. avium* and not *M. bovis* (Collins *et al.* 1985).

CONCLUSION

Tuberculosis 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 diagnose and treat tuberculosis cases. The diagnosis of tuberculosis in HIV patients is difficult for many reasons. Present study revealed evidence of tuberculosis infections was positive for 100 cases but, out of 100 positive causes there were 27 culture positive cases. 26 isolates were identified as Mycobacterium tuberculosis which was associated with HIV seropositivity. Only one isolate of *M. avium* complex (MAC) was found but it is not associated with HIV seropositivity. The lower isolation rate of *M. avium* complex (MAC) can probably be due to geographical variation.

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