CHAPTER - 6
DISCUSSION
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Tuberculosis is a chronic bacterial infection caused by *M. tuberculosis* and characterized by the formation of granulomas in infected tissues. The usual site of disease is the lung, but other organ may be involved. It is a disease of great antiquity and has caused more suffering and death than any other bacterial infection. Despite the availability of effective chemotherapy, it is still a major health problem in most countries of the world.

*M. tuberculosis* is transmitted from person to person via the aerial route. Tubercle bacilli in respiratory secretion form droplet nuclei and expelled during coughing, sneezing and vocalizing which gain access to the body. (Bass *et al*, 1990).

Tuberculosis usually causes symptoms. However, many patients, even some with extensive disease, have insidious symptoms that are commonly ignored. Other patients may be truly asymptomatic who can be identified only through a history of exposure. an abnormal chest radiograph, a positive reaction to a tuberculin skin test and cultures positive for tubercle bacilli.

The contribution of the microbiology laboratory to the diagnosis and management of tuberculosis involves the detection and isolation of *Mycobacteria*, identification of the *Mycobacterial* species and the determination of susceptibility of the organism to anti-mycobacterial
drugs. Microscopy is a simple and rapid means of detecting pulmonary tuberculosis patients. Methods for selective staining of *Mycobacteria* are the conventional acid-fast stain (Ziehl Neelsen) and the fluorochrome procedure, which uses Auramine O stain (Bass *et al.*, 1990). The sensitivity of microscopy depends primarily upon the number of bacilli in the specimen. More than 10,000 bacilli per ml sputum are necessary to secure microscopic positivity (Kim, *et al.*, 1984) but other factors also make the results of microscopy highly variable (22-96%) (Boyd and Marr, 1975; Narain, *et al.*, 1971; Blair, *et al.*, 1976.), though most authors put it around 60%. In our study, smear positivity was 63.44% - 75.44% and specificity was 100%, when compared to culture on LJ medium. It is well established that patients with sputum that is positive on direct smear examination are the principal sources of infection. But, definitive diagnosed is reached only by isolating the causative organism in culture. Further, in order to help the clinician in choosing the most effective anti-tuberculous agent and in the appraisal of the patients response to chemotherapy drug susceptibility test for *M. tuberculosis* have to be performed.

6.1 Age And Sex Distribution

The age of the pulmonary tuberculosis patients varied from 10 to 70 years (Table II) 66.3 percent of male patients were in the 21 to 40 years age group and 55.4 percent females were in 21 to 40 years age group.
According to Park and Park 1991, tuberculosis can occur at any age in India. The prevalence as well as incidence is higher as the age advances.

The male to female sex distribution in the study was 1.7:1 (Table II). Controls were similarly distribution (Table III). The male to female ratio ranging from 2:1 to 5:1 (Park and Park, 1991) have seen reported.

6.2 **Direct Smear Examination**

The detection of acid-fast bacilli in stained smears examined microscopically is the first bacteriologic evidence of the presence of *mycobacteria* in a clinical specimen. Sixty-six percent (200 Patients/300) patients were detected by Ziehl Neelsen smears and 63.44 percent sputum samples (571 sputum samples out of 900) were positive for acid-fast bacilli (Table XII) This is similar to 50 to 80 percent sputum smears positivity reported in patients with pulmonary tuberculosis (Bass, et al, 1990; Garay, 2000; Habeenu, et al., 1998; Placios, et al., 1997). The remaining 35 patients whose sputum sample did not reveal the presence of acid-fast bacilli by Ziehl Neelsen smear nevertheless had clinical and radiological evidence of tuberculosis. This cannot be attributed to inappropriate collection of specimens or delay in transport, as samples were collected under direct monitoring. It is well established that the frequency of excretion of acid-fast bacilli in pulmonary tuberculosis is intermittent, necessitating repeat examination of consecutive sputum samples. Perhaps the number of sputum samples
thus examined should be in excess of three to increase the percent of sputum acid-fast bacilli positivity in clinically and/or radiologically diagnosed patients of pulmonary tuberculosis. Alternatively, these patients may have received some form of intermittent anti-tubercular treatment not elicited in history, leading to cessation of bacilli in the sputum. Among the sputum smear positive patients, 10 patients had past history of receiving anti-tubercular treatment.

All smears positive by Ziehl Neelsen staining were also positive by Auramine O staining. Flourescent microscopy detected 71.33 percent (214 Patients/300) patients and 69.33 percent sputum samples (624 sputum samples out of 900) were positive (Table XII). Additional 5.9 percent sputum samples were positive by Auramine O staining (Table XII) which is slightly less than 2.77 percent additional sputum samples reported positive by Truant et al, 1962. Thus the result shows clear superiority of fluorescent staining over Ziehl Neelsen staining. Apart from higher positivity, another very important advantage of fluorescent microscopy is time saving. The average time devoted to screen Ziehl Neelsen stained of smear for AFB can is at ten minutes where as AFB can very easily be detected in fluorescent microscope within 2 minutes. Thus in a busy laboratory fluorescent microscopy is the preferred method.

It is well established that more patients of pulmonary tuberculosis can be diagnosed by repeated sputum examination. Additional 14 and 17 patients were positive for acid-fast bacilli by second and third
specimens, respectively (Table VII) which is similar to a study by Seetha et al, (1990). Some workers have reported a higher yield with second sputum examination (Aneja et al, 1979). Among 209 sputum positive patients only 172 patients had all 3 sputum specimens positive for acid fast bacilli (Table VI) reflecting the phenomenon of intermittent positivity (Nagpaul et al, 1974).

Attempts have been made to improve the efficacy of sputum smear examination by using sputum concentration techniques, the popular method being Petroff's technique. Additional 5.8 percent sputum smear positivity after Petroff's concentration has been reported over Ziehl Neelsen smears (Vasanthakumari, 1988). In the present study, on comparing with Ziehl Neelsen staining, additional 1.6 percent were positive by Auramine O staining and 4.2 percent after Petroff's concentration (TableX).

6.3 Culture on LJ Slopes

For definitive diagnosis of pulmonary tuberculosis, M. tuberculosis has to be isolated on culture. Culture is more sensitive than microscopy. Additional 23 patients (9.3 percent) were diagnosed by culture only (Table XIII) which is comparable to other studies (Nagpaul et al, 1974). Growth of the organism is also necessary for species identification. Two hundreded seventeen mycobacteria isolated in culture were identified as M. tuberculosis and twenty eight Mycobacteria isolated in culture were
identify as NTM on the basis of colony morphology, smear examination and biochemical tests. Among 227 sputum smear positive patients, 222 were also culture positive while, 5 (2.2 percent) were culture negative. This high percentage of 'smear only positives' not confirmed by culture were usually regarded as either false results or dead bacilli on account of previous treatment (Nagpaul et al, 1974). In the present study, it seems that in all the 5 patients, the bacilli were non viable and could not grow in culture as all the 5 patients had given history of taking antitubercular treatment recently.

Inoculated Lowenstein Jensen slopes were incubated for upto 8 week. Majority of the strain grew with in 4 weeks, 97.5 percent were positive by six week. Six additional isolates were obtained after 7 weeks of incubation and no additional isolates obtained in eight-week incubation (Table XIV). Various studies show that maximum growth occurs by 4 weeks of incubation (Topley and Wilson, 1990).

6.4 ISOLATION OF NTM

In the present study, the rate of isolations of NTM from patients suffering from various respiratory symptoms was 7.0% of total samples tested, and not changed much over the last twenty years (Bose, et al 1996). However, there is variability in the percentage of NTM out of total AFB positive culture. Shrinivas and Bhatia (1973) reported rate of 18.6% of total culture positive where as Bose (1996) reported a rate of
12.82% and Venkat Ram Shanker (1987) reported a rate of 5.87%, Shankar work was based on a local clinic catering primarily to tuberculosis patient and the low rate of isolation of NTM in that study may be due to this reason. In the presented study NTM accounted for 11.42% of the total positive cultures, overall increases in rate of isolation of NTM belonging to group-I and group-II. Chandrasekhar (1973) had no Runyon group-I NTM were in a study conducted around the same time. Shrinivas and Bhatia (1973) reported 14.36% of NTM isolated as belonging to Runyon group-I. Shankar based in a more definite group, reported 4.16% of his isolates (NTM) as belonging to Runyon group-I. On the other hand Bose (1996) reported 18.45% of NTM isolates as belonging to Runyon group-I. Our finding of 25% of NTM isolates as belonging to Runyon group-I is still higher than 18.46% as reported by Bose M (1996). Most of our group-I isolates turned out to be \textit{M. kansasii}, (Table-XVI). Another important observation of our study is definite rise in the rate of isolation of NTM belonging to group-III, 35.6% as compared to that of Bose (33.73%) in 1996. Moreover, 10 out of 28 isolates belonging to MAIC, which are definitely pathogenic NTM and have been repeatedly isolated from patient who are suffering from pulmonary mycobacteriosis (Paramsivam \textit{et al}, 1985) for more than one year. With the backdrop of lurking fear of AIDS assuming epidemic proportion in India, this may be important observation. Since a recent report from Amritsar has cited maximum number of strain (4.6%) of NTM belonging to Runyon group-III
(Agarwal, et al, 1993). A lower rate of isolation (17.7%) of NTM belonging to Runyon group-II, comprising mostly of non-pathogens was observed in our study (Table-XVI). Only 31.3% of total NTM isolated belonged to the other potential non-pathogenic group (Runyon group-IV).

6.5 Drug Resistance

An increase in the prevalence of strains with primary drug resistance indicates the extent of risk of infection in the community, whereas an increase in the prevalence of acquired resistance reflects the limitations of treatment available. Our aim was to study resistance to antitubercular drugs prevailing in a clinical situation. These estimates cannot reflect the epidemiological situation of resistance in the community. The drug resistance pattern of M. tuberculosis as reported by various workers in India varies from 34.3 to 70 percent for Streptomycin (Gupta, 1963 and Menon, 1963), 3.6 to 83.4 percent for INH (Selken et al, 1960 and Mahapatra 1964), 7.8 to 18.7 percent for Ethambutol (Krishnaswamy et al, 1984 and Das et al, 1985), 0 to 33 percent for Rifampicin (Jain, 1992; Troesch, 1999 and Kekkaku, 2000) and nil for Pyrazinamide (Trivedi, 1988). Similar results were obtained in this study with resistance to INH being 52.8 percent, streptomycin 11.1 percent, Rifampicin 43.05 percent, Ethambutol 18.05 percent and nil for Pyrazinamide.
The initial resistance to Ethionamide, INH, Rifampicin, Streptomycin, PAS, Cycloserine, Kanamycin, Ethambutol, Ciprofloxacin and Ofloxacin were 17.95%, 17.14%, 15.5%, 13.9%, 11.8%, 11.2%, 9.8%, 5.3% 4.5% and 2.44% and nil for Pyrazinamide respectively. Acquired resistance to Cycloserine was 29.8%, Rifampicin was 27.34%, Ethionamide was 27.75% Kanamycin was 26.12%, INH was 35.5%, and Streptomycin was 20.8% (Table XIX).

These results were similar to earlier studies (Krishnaswami et al, 1964; Trivedi, 1988; Kothadia, 1984; Chandrasekaran, 1991 and Jain et al, 1992), except that initial resistance to Ethambutol (nil) and Streptomycin (9%) was quit low. A study at Bangalore (Chandrasekaran, 1992), which reported an initial resistance of 0.5% to Ethambutol and 4.76% to streptomycin, is in agreement with ours. This low resistance to Ethambutol may be imported because Ethambutol is now assuming great importance in antitubercular therapy. The initial resistance to Rifampicin (15.5%) observed in our study was much higher than reported in other studies. This can prove to be a setback for tuberculosis control programme in our country.

The factors that contribute to the emergence of drug resistant in *M. tuberculosis* are likely to be many; but it is clear that non-compliance with therapy, unmonitored domiciliary therapy and intermittent therapy have greatly contributed to the emergence of drug resistance of *M. tuberculosis*. This is supported by the fact that initial resistance and
acquired resistance was observed to occur with similar frequencies. Given the endemicity of tuberculosis in our country and given the slow progress achieved in the development of new drugs for the treatment of this disease, it may perhaps be necessary to do a reappraisal of National Tuberculosis Control programmes. The real problem would be the patients harbouring drug resistant bacilli serving as a source of infection to susceptible individuals. It is a moot point, if acquisition of infection with *M. tuberculosis* that is already resistant to one or more drugs by a susceptible individual, would provide the bacillus an ideal environment to amplify its resistance to more drugs at an enhanced pace during the treatment. Should this occur, it is but natural that the proportion of resistant isolates in the community would register an increase and the difference between initial and acquired resistance would progressively diminish, resulting in newer problems to health planners concerned with tuberculosis control programmes.