CHAPTER - 2

SCREENING OF PULMONARY TUBERCULOSIS PATIENTS FOR THE ISOLATION OF CO-INFECTIONING CANDIDIA PATHOGENS

2.1 Introduction

Pulmonary tuberculosis is prevalent among people with poor socio-economic status. Occupational situation, poor diet and exposure to unhygienic polluted situation predisposes them to tuberculosis bacterial infection. Such tuberculosis affected patients prefer to go to Government hospitals for free treatment. Negligence to follow the physicians’ prescription to take antibiotic drugs for a course, smoking, living continuously in unhygienic situation are the factors barring them from a total relief from tuberculosis and many of them become carriers. Such persons with chronic history of tuberculosis get many opportunistic fungal infections. One such opportunistic infection predominantly reported was Candidiasis (Khanna and Nath, 1977; Liv et al., 2003 and Naz and Tariq, 2004).

The prevalence of opportunistic mycoses has dramatically increased during the past few years. The etiological agents of which are otherwise incapable of causing disease in healthy individuals. These opportunistic fungi are potential pathogen in the immunocompromised patients, patients with some pre-existing disease and patients with a long history of antibiotics (Schell, 1995 and Khan and Chugh, 2000). The rate of opportunistic fungal infections in tuberculosis patients is also very high. The reasons for increased prevalence are: lowering of immune system due to tuberculosis and the use of antituberculosis drugs of non-specific action which
promote the growth and reproduction of the fungus flora and in turn aggravate the course of underlying process in the lung tissues (Sain, et al., 1991 and Solov’eva, et al., 1991). Among the fungal pathogens, *Candida albicans* is a common yeast isolated from tuberculosis patients and it is responsible for causing severe secondary infections in such patients (Pukhlik, et al., 1990). Besides, a syntropic relationship between *C. albicans* and *Mycobacterium tuberculosis*, has also been reported in a number of studies where tubercle bacilli enable *C.albicans* to grow on Lowenstein Jensen’s medium, an inhibitory medium for *C. albicans* (Mankiewicz, 1954, 1957 and Mankiewicz, et al., 1959). Moreover, *C. albicans* also stimulated the growth of *M. tuberculosis* of reduced viability (Mankiewicz, 1954). Another study confirmed the effect of polysaccharide fraction of *C.albicans* for the enhancement of the growth as well as reduction of the generation time of tubercle bacilli (Ghafoor, 1967).

The stimulant action of *C. albicans* and their polysaccharide fraction has also been observed in vivo where the test animals died within weeks due to generalized tuberculosis when inoculated with both *M. tuberculosis* and *C. albicans*. However, the control animals, inoculated with only *M. tuberculosis* died after 6 weeks (Mankiewicz and Livak, 1960).

Keeping in view the role of Candida in aggravation of tuberculosis, the present study was undertaken to determine the prevalence of Candida infections among tuberculosis patients.
2.2 MATERIALS AND METHODS

2.2.1 Collection of sample

For sample collection, patients visited Government hospital with symptoms of tuberculosis was selected. A total of 500 patients were used for the study. Oral swabs (Hi-media) were used for taking the oral samples. As soon as the collection of sample was over, it was kept in an ice cold box and transported to the processing laboratory for isolation and characterization. The collection was done with the consent of the patients. The consent form has all the details of the patients. (Annexure. 1)

2.2.2 Collection of oral samples from tuberculosis patients

The patients were asked to wash their mouth with tap water for twice. Using the tongue depressor the tongue was pressed down and the lesions were noted in the oral cavity. The sterile swabs were gently applied and rotated in the lesions without touching the other parts of the cavity in order to avoid the contamination of other organisms. As soon as the collection was over it was immediately placed in the container and it was kept in an ice cold box to avoid further contamination and multiplication of micro organisms (Cheesbrough, 1994 and Patrick, et al., 2007). From the samples Candida species were isolated and identified using biochemical tests. The isolates were used for further study.

2.2.3 Gram's staining

The Gram staining was done as per the standard procedure (Collee, et al., 2003)
2.2.4 **Inoculation on SDA**

Sabouraud’s dextrose agar (Hi-media) agar was prepared by suspending 21.36 g of the medium in 500 ml distilled water, and boiled for completely dissolving the components. It was sterilized by autoclaving for 15 minutes, and dispensed in to the sterile petriplate. After complete solidification of the medium, the samples were swabbed. The plates were incubated at 37°C for 24 to 48 hours. After incubation, the plates were noted and recorded for the growth and colony morphology of the organisms.

2.2.5 **Inoculation on Hi-Chrom Candida agar**

Hi-Chrom (Hi-media) *Candida* agar was prepared by suspending 21.36g of the medium in 500ml distilled water, and steam sterilized. The medium was dispensed into sterile petriplates at ear lobe bearable temperature. The samples were inoculated and the plates were incubated at 37°C for 48-72 hours. After incubation, the plates were examined for the growth, colony morphology and pigment production of the organisms.

2.2.6 **Carbohydrate Fermentation Test**

Carbohydrate fermentation indicator broth medium was prepared in sterile test tubes with immersed Durham’s tubes. After being sterilized by autoclaving, 0.3ml of the filter sterilized carbohydrate solution (glucose, maltose, sucrose and lactose) was added aseptically. The yeast cultures with controls were inoculated into the appropriately labelled carbohydrate broth tubes under sterile condition and incubated at 30-37°C for 24-48hrs. As shaking of the tube may accidentally force a bubble of air into the inverted Durham’s tube, displacing the medium and possibly
rendering a false positive result, care was taken not to shake the fermentation tube while inoculation. (Baron, et al., 1994).

2.2.7 Carbohydrate Assimilation Test

Carbohydrate Assimilation Media - Yeast Nitrogen Base Agar was prepared by adding filter sterilized 10 ml of Yeast Nitrogen Base solution to 90ml of 2% sterilized agar and poured into the sterile petriplates. Sugar discs were prepared by placing Whatmann No: 3 filter paper discs into each of the filter sterilized carbohydrate solution (20% - glucose, maltose, sucrose and lactose) and dried. The surface of the medium was covered with the suspension of yeast cells and was allowed to dry. The sugar soaked filter paper discs were placed in the designated area using sterile forceps. The plates were incubated at 30-37°C for 24-48 hours. After incubation, the plates were observed for the assimilation of carbohydrates by growth of the organisms around the sugar disc (Baron, et al., 1994).

2.2.8 Germ Tube Test

A loopful of yeast culture was inoculated into 0.5ml of human serum taken in a small glass test tube and incubated in a water bath at 37°C for 2 hours. After incubation, a wet mount of a drop of yeast serum mixture was prepared and examined under low power objective of the microscope for germ tube formation (budding yeasts with long projection like growth). The positive and negative controls used were *C. albicans* and *C. parapsilosis* respectively (Jawetz, et al., 1987).

* Details of material used are given in annexure-2.
2.3 RESULTS AND DISCUSSION

In recent years, fungal infections are under rise, due to various predisposing factors such as patients on steroid, long term administration of antibiotics, HIV infection and many of them have basic pulmonary disease like tuberculosis which may also alter the quotes of the disease. Ahearn, et al., (1966) Jain, et al., (1982) and Khanna et al., (1977) reported opportunistic mycoses associated with pulmonary tuberculosis due to the deficiency of host resistance.

In the present study the co-existence of candidiasis was reported from 500 cases of pulmonary tuberculosis patients. All these patients belonged to different age group and sex. Out of the 500 cases screened, the number of cases positive for the co-existence of opportunistic Candida infection and pulmonary tuberculosis were 15 females and 79 males. Oral swabs were taken from the 79 positive males and 15 positive females. The oral swabs from the pulmonary tuberculosis infected patients revealed the presence of different types of Candida spp. (Table.1). The Candida spp. isolated were C. albicans, C. tropicalis and C. glabrata. Of the three species C. albicans was predominant in males (11.2%) and less in females (3.6%). C. tropicalis registered a high rate of infection (8.3%) in males and (3.1 %) in females. Out of the 79 patients screened 5.8% males were positive for C. glabrata. In females the incidence of C. glabrata infection was comparatively less (1%).

From the result, it is clear that the C. albicans is a major causative agent for candidiasis in pulmonary tuberculosis patients. Jain, et al., (1982) reported Candida infection in 33 patients out of 140 pulmonary tuberculosis patients screened. Pandalai and Kurup (1962) isolated C. krusei (2.9%) and C. parapsilosis (8.7%) out of 69 cases of pulmonary tuberculosis patients. Naz and Tariq (2004) reported the
presence of *Candida* co-infection in tuberculosis patients screened. Of these, *C. tropicalis* ranked high with an incidence rate of 8.4% as compared to *C. albicans* 6.8% (34/500). In the present study 18.8% cases positive for co-infection of tuberculosis and candidiasis had *C. albicans*’s infection. This finding is parallel to the report of Liv, et al., (2003) and Eggiman, et al., (2003).

The sex wise distribution of the co-infected patient exhibited high trend of *Candida* infection among male tuberculosis patients (25.3%; 79/310) as compared to female tuberculosis patients (7.7%; 15/190). The relationship between positivity of co-infection and sex further showed that out of 94 positive cases, 84% were males 16% were females and in the 406 non co-infected cases, 57% were males and 43% were females (Table. 2). As far as the sex-wise distribution of *Candida* infection, it was evident from literature that the colonization with *Candida* species occured in equal numbers in males and females (Hidalgo and Vazquez, 2004).

However, in the present study *Candida* infections were found more prevalent in male tuberculosis patients as compared to female. This might be attributed more to the exposure of males to external environment and also due to the habits of smoking (Murray, 1992) and social stigma (Rajeswari, et al., 1999). The higher rate of co-infection of Candidiasis in male was observed due to their smoking habit and alcoholism.

In the present study the patients identified with tuberculosis were in the age group of 30-50 years. Among them the co-infection rate was high in the age group 41-50 years (76.6%). In the age group 30-40 years, 23.45% were found with the co-infection of *Candida* and tuberculosis (Table.3).
The predominance of the co-infection in the age group 41-50 was due to the chronic problem of their respiratory system as the age of the person increased and treatment of such infection for a long time led to the weakening of the immune system. When the immune system gets weakened, it paves way for opportunistic Candida co-infection. Chi square test confirmed the relationship between the age group of individual and the co-infection.

**Table 1. Co-existence of different species of Candida in tuberculosis infected patients.**

<table>
<thead>
<tr>
<th>Name of the organisms</th>
<th>Candida infection in patients screened</th>
<th>No. Candida infected causes in total patients screened</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Male n=310</td>
<td>Female n=190</td>
</tr>
<tr>
<td></td>
<td>No.</td>
<td>%</td>
</tr>
<tr>
<td>Candida albicans</td>
<td>35</td>
<td>11.2</td>
</tr>
<tr>
<td>Candida tropicalis</td>
<td>26</td>
<td>8.3</td>
</tr>
<tr>
<td>Candida glabrata</td>
<td>18</td>
<td>5.8</td>
</tr>
<tr>
<td>Total</td>
<td>79</td>
<td>25.3</td>
</tr>
</tbody>
</table>
Table. 2. Chi-square test for the number of co-infected and non co-infected cases of Candidiasis and tuberculosis

<table>
<thead>
<tr>
<th>Sex</th>
<th>No. of patients co-infected</th>
<th>No. of patients not infected</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>79 (84%)</td>
<td>231 (57%)</td>
<td>310</td>
</tr>
<tr>
<td>Female</td>
<td>15 (16%)</td>
<td>175 (43%)</td>
<td>190</td>
</tr>
<tr>
<td>Total</td>
<td>94</td>
<td>406</td>
<td>500</td>
</tr>
</tbody>
</table>

Calculated value of Chi-square = \( \sum (O-E)^2/E = 23.74 \).

Null hypothesis is rejected. There is significant relationship between gender and positivity of infection.

Table. 3. The relationship between age and co-existence of Candida and tuberculosis

<table>
<thead>
<tr>
<th>Age</th>
<th>No. of patients co-infected</th>
<th>No. of patients not infected</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>30-40</td>
<td>22</td>
<td>243</td>
<td>265</td>
</tr>
<tr>
<td>41-50</td>
<td>72</td>
<td>163</td>
<td>235</td>
</tr>
<tr>
<td>Total</td>
<td>94</td>
<td>406</td>
<td>500</td>
</tr>
</tbody>
</table>

Calculated value of Chi-square = \( \sum (O-E)^2/E = 40.7 \).

Null hypothesis is rejected. Age and positivity of infection are dependent.