Frequency and Pattern of Pulmonary Fungal Infections in Patients with Pulmonary Tuberculosis

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The present study was designed to find out the frequency and pattern of pulmonary fungal infections in patients with pulmonary tuberculosis. Two hundred sputum samples were taken of patients with pulmonary tuberculosis. Out of 200 sputum samples, one hundred sputum samples were of freshly diagnosed patients with pulmonary tuberculosis and one hundred patients on antituberculous drugs for at least three months. Fifty sputum specimens from healthy attendants were also examined as controls. All sputum specimens were examined for fungal hyphae and spores by microscopic examination with 10% potassium hydroxide, lactophenol, cotton blue stain and calcofluor white stain (fluorescent staining). Culture on Saboraud's medium with chloramphenicol was also used for isolation of fungi from sputum specimens. Out of 200 sputum specimens 32 (16%) were positive for fungal hyphae and spores with 10% potassium hydroxide and lactopherol cotton blue staining. While 54(27%) sputum samples were positive for fungus with calcofluor white staining technique and 68(34%) were positive with culture technique. While no specimen was positive for fungus by any technique among controls. The positivity was significantly higher among tuberculous patients on treatment as compared with freshly diagnosed patients (P<0.001), among patients with duration of illness more than one year as compared with less than one year (P<0.001), patients with cavitary lesions on X-rays and history of haemoptysis (P<0.001). The most frequent fungal isolate was Candida tropicalis (n=37) followed by Candida albicans (n=17) and Aspergillus fumigatus (n=12). Based on these findings, it is suggested that sputum specimens of patients with tuberculosis should be routinely examined for the presence of fungi (especially with cavities and haemoptysis).

Key word: Pulmonary fungal infections, Aspergillus, Pulmonary tuberculosis

Pulmonary fungal infections have emerged as an important cause of morbidity and mortality. Opportunistic fungi grow in the cavities which are produced by chronic pulmonary tuberculosis.[1,3] These co-existing fungal infections increase the activity and virulence of the tuberculous process. They may not only affect the course of disease process but also hamper the effect of usual anti-tuberculous treatment. Thus most of these patients are branded as resistant to anti-tuberculous treatment. The co-existance of pulmonary tuberculosis and fungal diseases is a well documented feature[4].

Cavities and cystic lesions of the lung which communicate with the airways are poorly cleared of secretions and inhaled particulates. Therefore such lesions are susceptible to saprophytic colonization by fungi such as Aspergillus, whose spores circulate in the environmental air. Healed tuberculous cavities and other post inflammatory spaces are colonized in this fashion[5].

There are nine important pulmonary mycoses. These are classified into two major groups, endemic and opportunistic. The endemic pulmonary mycoses include cryptococcosis, histoplasmosis, blastomycosis, coccidioidomycosis and paracoccidioidomycosis. These infections can occur in persons with intact immunity. However opportunistic fungal infections occur in persons with depressed immunity. These include aspergillosis, mucormycosis and candidiasis[6]

As already mentioned pulmonary tuberculosis is a very common public health problem in our country. These patients are treated with anti-tuberculous drugs. Some of these patients continue to have clinical and radiological evidence of chronic pulmonary infection due to ineffective or inadequate treatment. Another reason of persistent pulmonary symptoms in these patients may be coexisting fungal infections. In some cases physicians do prescribe antifungal drugs but only on emprical basis. However there is no published data about the role of fungi in patients with pulmonary tuberculosis. The present study was planned to determine the frequency and pattern of fungal pulmonary infections in patients with pulmonary tuberculosis. This will be helpful in creating awareness about the role of fungi in these patients and better management of the patients by the physicians.

Patients and methods

The present study was carried out on two hundred and fifty subjects. Out of these, two hundred subjects were suffering from pulmonary tuberculosis. These patients were selected from Gulab Devi Hospital, Lahore irrespective of age and sex. Diagnosis of pulmonary tuberculosis was based on sputum smear positivity for acid fast bacilli by Zeihl Neelson staining technique[7]. Out of the two hundred patients with pulmonary tuberculosis, one hundred were freshly diagnosed cases. They had either not received anti-tuberculous treatment or were on treatment for less than one week. The rest of 100 patients were already diagnosed cases of pulmonary tuberculosis. They had been on anti-tuberculous treatment for at least three months. Fifty age and sex matched healthy contacts (attendants of the

patients) were included as controls.

Early morning sputum specimens were collected from each patient and control. For this purpose three sterile screw capped, wide mouthed, leak proof, properly labelled containers were provided to the patients and controls. Patients were instructed to collect the first morning sputum sample before breakfast. They were asked to rinse their mouth with water vigorously, cough out the sputum sample directly into the container and recap it. The samples were then transported to the laboratory within 2 hours of collection. Each specimen was examined for the presence of fungal hyphae and spores by microscopic examination with ten percent potassium hydroxide, lactophenol cotton blue stain and calcofluor white staining. All specimens were also inoculation on Sabouraud's medium with chloramphenicol for isolation of fungi[8,9].

Results

Table 1 and table 2 show that out of one hundred freshly diagnosed patients with pulmonary tuberculosis, four sputum samples were positive for fungus with ten percent potassium hydroxide and lactophenol cotton blue techniques. These tables also show that out of one hundred patients on anti-tuberculous treatment, twenty-eight sputum samples were positive for fungus with ten percent potassium hydroxide and lactophenol cotton blue techniques. Out of controls no sputum sample was positive for fungus. Statistical analysis revealed that positivity for fungus was significantly higher among patients on anti-tuberculous treatment (P<0.001) as compared with freshly diagnosed patients and controls.

Table 1. Distribution of patients with tuberculosis and controls according to results of sputum examination by 10% potassium hydroxide preparations

Group	Result of potassium hydroxide preparation	
	Fungus +ve	Fungus -ve
Freshly diagnosed patients (n=100)	4*	96
Patients on antituberculous treatment (n=50)	28**	72
Controls (n=50)	0	50

Figures in parentheses indicate the number of cases in each category
*P>0.05 Not significant as compared with controls
**P<0.001 Significantly higher as compared with freshly diagnosed tuberculosis patients and controls

Table 2. Distribution of patients with tuberculosis and controls according to results of sputum examination by lactophenol cotton blue staining.

Group	Result of lactophenol cotton blue staining	
	Fungus +ve	Fungus -ve
Freshly diagnosed patients (n=100)	4*	96
Patients on antituberculous treatment (n=50)	28**	72
Controls (n=50)	0	50

Figures in parentheses indicate the number of cases in each category
*P>0.05 Not significant as compared with controls
**P<0.001 Significantly higher as compared with freshly diagnosed tuberculosis patients and controls

Table 3 and table 4 show a comparison of fungus positivity among patients and controls by calcofluor white staining technique and culture methods. On calcofluor staining thirteen patients were positive for fungus among freshly diagnosed patients. While forty-one patients were positive among patients on anti-tuberculous treatment. Among controls, fungus was not observed in any sputum sample. On culture thirteen freshly diagnosed cases of tuberculosis were positive for fungus while 55 patients on anti-tuberculous treatment were positive. Using both these techniques, significantly higher number of freshly diagnosed patients (P<0.05) and patients on anti-tuberculous treatment (P<0.001) were positive for fungus as compared with controls.

Table 3. Distribution of patients with tuberculosis and controls according to results of sputum examination by calcofluor white staining.

Group	Result of calcofluor white staining	
	Fungus +ve	Fungus -ve
Freshly diagnosed patients (n=100)	13*	87
Patients on antituberculous treatment (n=50)	41**	59
Controls (n=50)	0	50

Figures in parentheses indicate the number of cases in each category
*P<0.05 Significantly higher as compared with controls
**P<0.001 Significantly higher as compared with freshly diagnosed tuberculosis patients and controls

Table 4. Distribution of patients with tuberculosis and controls according to results of sputum examination by culture.

Group	Result of culture	
	Fungus +ve	Fungus -ve
Freshly diagnosed patients (n=100)	13*	87
Patients on antituberculous treatment (n=50)	55**	45
Controls (n=50)	0	50

Figures in parentheses indicate the number of cases in each category
*P<0.05 Significantly higher as compared with controls
**P<0.001 Significantly higher as compared with freshly diagnosed tuberculosis patients and controls

Discussion

Pulmonary fungal infections are particularly common in the presence of cavities and cystic lesions of lungs which communicate with the airways. Fungi colonize these lesions and start infections[5].These fungal infections cause a variety of inflammatory reactions. These include epithelial hyperplasia, histiocytic granuloma and thrombotic arteritis. Usually there is a mixed pyogenic and granulomatous picture with fibrosis and calcification[10].

Most of the commonly used antibiotics have adverse effects on the immune response of human body. They tend to disturb chemotaxis, lymphocyte transformation, delayed hypersensitivity and antibiody production[11]. In a host with intact immunity, pathogenic fungi which cause systemic infections are controlled or eradicated from the body by the cell mediated immune response of the host[12]. However in immunosuppressed patients opportunistic fungal infections have become important cause of sickness and death[13]. Patients with tuberculosis are treated with antituberculous drugs like rifampicin, ethambutol, pyrazinamide and streptomycin. These drugs also have

adverse effects on the immune system of tuberculous patients[11]. It is quite evident from the results of the present study that pulmonary fungal infections are significantly higher (P<0.001) in these pulmonary tuberculosis patients who had been on antituberculous treatment.

Out of 200 tuberculosis patients examined in the present study, sixty-eight (34%) were positive for fungus on culture. This finding clearly indicates that tuberculosis patients commonly have coexisting fungal infections of lungs. Such coexistence has been observed by many workers abroad[1,3,4].

According to a study carried out by Tomlinson and Sahn[4] out of 28 patients with aspergillosis, fourteen had tuberculosis, thus stressing the importance of coexistence of pulmonary fungal infection and tuberculosis.

Another study on coexistence of fungal infections in patients with pulmonary tuberculosis was also carried out in India by Jain et al[4] Fresh morning samples of sputum were collected from 140 sputum positive pulmonary tuberculosis patients from the department of tuberculosis and chest diseases. The specimens were examined for fungus by making smears and performing fungus culture. Seventy-four cases (52.85%) showed positivity for fungus both by direct smear and culture examinations. This figure is higher than observed in the present study. In this study, thirty-four percent patients were positive for fungus on culture. While twenty-seven percent patients were positive on microscopy by fluorescent staining technique.

Common fungi responsible for pulmonary fungal infections have been observed to be Candida and Aspergillus species by many workers[6,14,15]. In the present study, all the fungal isolates belonged to Candida and Aspergillus species. Therefore, the results of present study are consistent with the results of studies by these workers.

Candida species were isolated from significantly higher (P<0.01) and (P<0.001) number of patients in freshly diagnosed and treated cases of tuberculosis respectively as compared with Aspergillus species. Population based active laboratory surveillance for invasive mycotic infections was conducted during 1992 and 1993 in three California counties by Rees et al[15] In their study, invasive mycoses were most commonly caused by Candida followed by Cryptococcus, Coccidioides, Aspergillus and Histoplasma.

Candida albicans is considered to be the most common candida species responsible for candidiasis[16]. Candida tropicalis is the second commonest candida species isolated from different clinical specimens[5,16] In the present study, candida species were isolated from specimens of 27 percent patients with tuberculosis. Candida tropicalis was the major isolate followed by candida albicans. This finding is different from findings of other workers[17,18,19]..

Among Aspergillus species Aspergillus fumigatus is the most common fungal pathogen[15] which is consistent with our study. These findings are in agreement with those of other workers[4,15,20,21]..

Fluorescent microscopy using calcofluor white dye has been used by many workers for the demonstration of fungal hyphae and spores in respiratory secretions[22].

Conclusion

It can be concluded that coexisting pulmonary fungal infections are not uncommon in patients with pulmonary tuberculosis. Fungal infections were observed more often in patients with history of haemoptysis and with cavitary lung lesions. For diagnosis of pulmonary fungal infections, calcofluor white staining is a rapid method with high positivity yield. Moreover, for definite diagnosis, fungal cultures should always be applied to correlate with findings on direct microscopy. Hence sputum specimens of all patients with pulmonary tuberculosis should be tested for the presence of fungi. This will help in getting knowledge about the pattern of fungi responsible for coexisting fungal infections in these patients as well as proper management.

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