Pak. J. Bot., 36(4): 857-862, 2004.

A STUDY OF THE TREND IN PREVALENCE OF OPPORTUNISTIC CANDIDAL CO-INFECTIONS AMONG PATIENTS OF PULMONARY TUBERCULOSIS

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Abstract

Five hundred clinical specimens of sputa, bronchial aspirations, and pleural effusions were collected from hospitalized tuberculous patients for the isolation of *Candida* species. The patients were categorized in two groups. Group A included tuberculous patients having some complications like nonsubsiding fever, marked cough and persistence of other symptoms in spite of taking antituberculous treatment. Group B included turberculous patients having no complications. The *Candida* species were isolated and identified on the basis of morphological, cultural and biochemical characteristics. *Candida* species were isolated from 15.2% (76/500) specimens. The incidence rate of Candidal co-infection was higher in Group A patients (16.1%) as compared to Group B patients (13.8%). Among the *Candida* species, *Candida tropicalis* (8.4%) predominated over *Candida albicans* (6.8%). Furthermore, the incidence of Candidal infection was higher in male patients (16.3%) as compared to female patients (13.9%).

Introduction

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 immuno-compromised patients, patients with some pre-existing disease and patients with a long history of antibiotics (Schell, 1995; Khan & Chugh, 2000). The rate of opportunistic fungal infections in turberculous patients is also very high. The reasons for increased prevalence are lowering of immune system due to tuberculosis and the use of antituberculous 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; Solov'eva *et al.*, 1991).

Among the fungal pathogens, *Candida albicans* is a common yeast isolated from tuberculous 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 were found to enable *C.albicans* to grow on Lowenstein Jensen's medium, an inhibitory medium for *C. albicans* (Mankiewicz, 1954, 1957; Mankiewicz *et al*, 1959). Moreover, *C. albicans* also stimulated growth of *M. tuberculosis* of reduced viability (Mankiewicz, 1954). Another study confirmed the effect of polysaccharide fraction of *C. albicans* for 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

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6 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 & Liivak, 1960).

Keeping in view the role of *Candida* in aggravation of tuberculosis, the present study was undertaken to determine the prevalence of Candidal infections among tuberculous patients.

Materials and Methods

The study comprised of 500 tuberculous patients, admitted to Ojha Institute of Chest Diseases, Karachi. These patients were further categorized in two groups on the basis of their clinical findings and physician's recommendations.

Group A: Included tuberculous patients having some complications like no subsiding fever, marked cough, hemoptysis, raised ESR and leucocytosis in spite of taking antituberculous drugs.

Group B: Included tuberculous patients having no complication.

Collection of specimens: Clinical specimens; sputa, bronchial aspiration and pleural effusion were collected from the patients.

Direct smear examination: All the clinical specimens were Gram stained and observed under the microscope for the yeast cells.

Culture for primary isolation: Sabouraud dextrose agar was used for primary isolation of *Candida* species. The culture plates were incubated at 37^{o} C for 24 hours.

Characterization of organisms: All pure cultures were characterized to species level using different tests conforming with required standard diagnostic criteria (Baron *et al.*, 1994; Cheesbrough, 1994). The criteria included morphological and cultural characteristics, Germ tube experiment and tests for carbohydrate assimilation and fermentation.

Results

A total of 500 tuberculous patients (AFB positive); 270 males and 230 females were included in the study. The clinical specimens collected from these patients were processed for the isolation of *Candida* species. The study revealed 15.2% (76/500) of the total tuberculous patients co-infected with *Candida* species. Of these, *C. tropicalis* ranked high with an incidence rate of 8.4% (42/500) as compared to *C. albicans* (6.8%; 34/500). The sex-wise distribution of these co-infected patients exhibited higher trend of Candidal infections among male tuberculous patients (16.3%; 44/270) as compared to female tuberculosis patients (13.9%; 32/230) (Table 1).

The tuberculous patients showing some complications (Group A) exhibited 16.1% (49/304) co-infection with *Candida* species, where *C. tropicalis* was observed with higher incidence rate of 8.9% (27/304) as compared to *C. albicans* (7.2%; 22/304). The Group B patients (patients showing no complication) also revealed co-infection with *Candida* species with an incidence rate of 13.8% (27/196). The incidence of *C. tropicalis* was observed with higher incidence rate of 7.7% (15/196) as compared to *C. albicans* which exhibited 6.1% (12/196) incidence rate (Table 2).

Table 1. Prevalence of Candidal infections among tuberculous patients with respect to sex.

Organisms	Patients co-infected with *Candida* species				Total n=500	
	Male n=270		Female n=230			
	No.	%	No.	%	No.	%
Candida albicans	19	7.0	15	6.5	34	6.8
Candida tropicalis	25	9.3	17	7.4	42	8.4
Total	**44**	**16.3**	**32**	**13.9**	**76**	**15.2**

Percentages have been calculated from corresponding values of n.

Table 2. Prevalence of *Candida* species among tuberculous patients with respect to complications.

Candida species	Total no. of isolates	Group A		Group B	
		Patients with complications N=304		Patients without complications n=196	
		No.	%	No.	%
C. albicans	34	22	7.2	12	6.1
C. tropicalis	42	27	8.9	15	7.7
Total	**76**	**49**	**16.1**	**27**	**13.8**

Percentages have been calculated from corresponding values of n.

Discussion

Candida is a component of the normal microflora of the alimentary tract and mucocutaneous membrane of healthy host. However, the slight alteration in the physiological state can turn normally harmless commensal yeast into aggressive pathogen causing mucosal, superficial or even life threatening systemic infections in the immunocompromised host pointing to the pathogenic potential of *Candida* species (Newman & Holly, 2001; Hube & Naglik, 2001).

The prime target of these opportunistic Candidal infections are patients who are critically ill and are at medical and surgical intensive care units. *Candida* species are well recognized in nosocomial infections and have been reported as sixth most common nosocomial pathogen (Beck-Sagu'e & Jarvis, 1993). The high prevalence has also been reported in a study where the overall distribution of fungi causing nosocomial infection at hospitals from 1980 to 1990 revealed 72.1% fungal infections caused by *Candida* species (Jarvis, 1995).

The role of Candida species as secondary invader of lungs, kidneys and other organs of patients having some pre-existing disease like tuberculosis and cancer, have also been documented (Jawetz *et al*., 1987). The present study which was conducted to determine the incidence rate of Candidal infections among tuberculous patients admitted in hospital revealed 15.2% co-infection of tuberculous patients with *Candida* species. This finding is parallel with another study conducted in China which revealed 21.6% fungal infections among hospitalized patients having underlying primary pulmonary diseases. The main pathogen involved in these infections were *Candida* species (Liv, *et al*., 2003). The

similar pattern has also been observed from another study which reported 15% Candidal infections among non-immunocompromised critically ill patients (Eggiman *et al.*, 2003).

As far as the sex-wise distribution of Candidal infection is concerned, it is evident from literature that the colonization with *Candida* species occur in equal numbers of males and females (Hidalgo & Vazquez, 2004). However, in the present study Candidal infections were found more prevalent in male tuberculous patients as compared to females. This might be attributed to more exposure of male to external environment and their habit of using some addictive substances (Murray, 1992).

In the present study, the specie-wise distribution of Candidal infections revealed the predominance of *C. tropicalis* over *C. albicans*. Until recently, *C. albicans* has remained the principal etiologic agent in Candidiasis. However, over the past decade there has been a distinct change in the species of *Candida* associated with nosocomial infections as well as rise in the incidence of such infections. *C. albicans* which caused 80% of Candidal blood stream infections in 1984 was observed responsible for fewer than 50% of these infections in 1991. More infections have been reported with other *Candida* species (Wenzel, 1995). The proportion of non-albicans *Candida* species isolated from blood cultures actually equaled or exceeded that of *C. albicans* (Blinkhom, *et al.*, 1989). In a study conducted in Kuwait, the data of the last five years on yeast isolates suggest that *Candida* species other than *C. albicans* are most frequently associated with blood stream infections (Randhawa, 2000). This finding is supported by other studies which also revealed increasing proportion of blood stream infections caused by *Candida* species other than *C. albicans* (Pfaller, 1994; Hsueh & Chen, 2002).

Among non-albicans *Candida* species, *C. tropicalis* has been emerging as a new opportunistic pathogen to cause severe invasive disease. *C. tropicalis* has an apparently greater capacity than *C. albicans* to invade the deep tissues of immunocompromised host. Several studies have shown positive surveillance cultures of *C. tropicalis* to be highly predictive of subsequent systemic infection. The higher incidence of colonization with *C. albicans* as compared to *C. tropicalis* has also been observed in leukemic children. However, a higher proportion of those colonized by *C. tropicalis* developed fungaemia (Barnes & Wardley, 1996). Furthermore, the outbreaks of neonatal fungaemia by *C. tropicalis* in the neonatal intensive care units have also been documented in recent literature (Chowdhary *et al.*, 2003; Roilides *et al.*, 2003).

These Candidal infections, when are associated with pre-existing disease, may cause many complications in the primary disease. It has also been observed that secondary fungal infections in the lungs of pulmonary tuberculous patients are associated with marked cough, expectoration, dyspnea, fever, anaemia, leucocytosis and raised ESR (Jain *et al.*, 1991). The role of *C. albicans* in causing severe secondary infections in tuberculous patients has also been reported in a study where, in spite of successful completion of antituberculous chemotherapy, patients suffered from continued chronic cough, sputum or occasional hemoptysis (Kim *et al.*, 1988). These findings correlated with results of present study where 16.1% of the tuberculous patients were co-infected with *Candida* species and manifested different complications in their primary disease. Among the *Candida* species, *C. tropicalis* emerged as a predominant organism, which is also a noteworthy observation of the present study and provides an insight into the prevalence of Candidal infections among hospitalized tuberculous patients and indicate that these Candidal infections may enhance the primary disease such as tuberculosis. In addition, it is evident from the literature that 62.6% of the tuberculous patients having

oral Candidiasis were HIV positive (Ahmed *et al.*, 2003). Thus, the co-infection of tuberculous patients with Candida may provide a diagnostic or highly suspicious index for HIV infection too. However, the diagnosis of opportunistic respiratory fungal infections pose a difficult diagnostic challenge due to lack of any pathognomonic clinical syndromes. In developing countries like Pakistan these problems are further increased by preponderance of pulmonary tuberculosis which may result in unavoidable complications of unwarranted chemotherapy or surgery.

Acknowledgements

We are most grateful to Dr. Shahina Qayyum, Chief Medical Officer, Ojha Institute of Chest Diseases, Karachi, for valuable discussion and cooperation in collection of specimens from the hospital. We also extend our thanks to all medical and paramedical staff members of Ojha Institute who provided us all the facilities which we needed for collection of specimens from the hospital.

References

Ahmed, Z., R. Bhargava, D.K. Pandey and K. Sharma. 2003. HIV seroprevalence in tuberculosis patients. *Ind. J. Tub*., 50:151-154.

Barnes, A.J. and A.M. Wardley. 1996. Fatal *Candida tropicalis* fungaemia in leukaemic patients receiving fluconazole prophylaxis. *J. Infect*., 33: 43-45.

Baron, E.J., L.R. Peterson and S.M. Finegold. 1994. Laboratory methods in basic mycology. In: Bailey & Scotti's 9th Edition. *Diagnostic Microbiology*. The C.V. Mosby Company. 689-775.

Beck-Sagu'e, C.M. and W.R. Jarvis.1993. National nosocomial infections surveillance system. Secular trends in the epidemiology of nosocomial fungal infections in the United States 1980-1990. *J. Infect. Dis*., 167: 1247-51.

Blinkhom, R.J., D. Adelstein and P.J. Spagnulo. 1989. Emergence of a new opportunistic pathogen, *Candida lustaniae*. *J. Clin. Microbiol*., 27: 236-240.

Cheesbrough, M. 1994. Mycology. In: *Medical Laboratory Manual For Tropical Countries.* Tropical Health Technology/Butter Worth Heineman Ltd, ELBS Edition. 11: 372-391.

Chowdhary, A., K. Becker, W. Fegeler, H.C. Gugnani, L. Kapoor and V.S. Randhawa. 2003. An outbreak of Candidemia due to *Candida tropicalis* in a neonatal intensive care unit. *Mycoses*, 46(8): 287-292.

Eggimann, P., J. Garbino and D. Pittet. 2003. Epidemiology of *Candida* species infections in critically ill non- immunosuppressed patients. *Lancet Infect. Dis*., 3(II): 685-702.

Ghafoor, M. 1967. Effect of the polysaccharide fraction of *Candida albicans* on the growth of *Mycobacterium tuberculosis.* Thesis. Jinnah Post Graduate Medical Centre, Karachi, Pakistan.

Hidalgo, J.A. and J.A. Vazquez. 2004. Candidiasis. e - *Medicine Journal* 2004 5(3).

Hsueh, P.R. and M.L. Chen. 2002. Antimicrobial drug resistance in pathogens causing nosocomial infections at a University Hospital in Taiwan 1981-1999. *Emerg. Infect. Dis*., 8(1).

Hube, B. and J. Naglik. 2001. *Candida albicans* proteinases : resolving the mystery of a gene family. *Microbiol*., 147: 1997-2005.

Jain, S.K., R.L. Agrawal and R.C. Pandey. 1991. A clinico- radiological study of secondary mycoses in pulmonary tuberculosis. *Indian J. Med. Sci*., 45(4): 81-84.

Jarvis, W.R. 1995. Epidemiology of nosocomial fungal infection with emphasis on *Candida* species. *Clin. Infect. Dis*., 20(6): 1525-1530.

Jawetz, E., J.L. Melnick and E.A. Adelberg. 1987. *Medical Mycology*. In: *Review of Medical Microbiology*, 17th Edition, Appleton and Lange, Norwalk Connecticut/Los Altos, California, 318-337.

Khan, Z.U. and T.D. Chugh. 2000. Invasive fungal infections in Kuwait: A retrospective study. *Indian J. Chest Dis. & Allied Sci*., 42(4): 297-87.

Kim, S.J., Y.P. Hong and S.O. Kin. 1988. Fungal complications in patients with pulmonary tuberculosis or other lung disease. *Korean J. Mycol*., 16 (1): 26-32.

Liv, Z.Y., R.Y. Sheng, X.L. Li, T.S. Li and A.X. Wang. 2003. Nosocomial fungal infections, analysis of 149 cases. *Zhonghua Yi Xue Za Zhi*., 83(5): 399-402.

Mankiewicz, E. 1954. *Mycobacterium tuberculosis* and *Candida albicans*: A study of growth promoting factor. *Canad. J. Microbiol*., 1: 85-89.

Mankiewicz, E. 1957. *Candida albicans* a mean for detecting *M. tuberculosis on* culture media. *Am. Rev. Tuberc*., 75: 836-840

Mankiewicz, E. and M. Liivak. 1960. Effect of *Candida albicans* on the evolution of experimental tuberculosis. *Nature*, 187:250-251.

Mankiewicz, E., E. Stuckiewicz and M. Liivak. 1959. A polysaccharide isolated from *Candida albicans* as a growth promoting factor for *Mycobacterium tuberculosis. Canad. J. Microbiol*., 5(3): 261- 267.

Murray, C.J.L. 1992. *Draft trip report, Geneva.* WHO, CDS.

Newman, S.L. and A. Holly. 2001. *Candida albicans* is phagocytosed, killed and processed for antigen presentation by human dendritic cells. *Infect. & Immun*., 69(II): 6813-6822.

Pfaller, M.A. 1994 . Epidemiology and control of fungal infections. *Clin. Infect. Dis*., 19 (Supp 1): S8-13.

Pukhlik, B.M., S.V. Zaikov and I.V. Kornitskaya. 1990. Sensitization to *Candida* fungi in patients with tuberculosis. *Urach. Delo*., 0(11): 22-24.

Randhawa, H.S. 2000. Respiratory and systemic mycoses: An overview. *Indian J. Chest Dis. & Allied Sci.* , 42(4): 207-19.

Roilides, E., E. Farmaki, J. Evdoridou, A. Francesconi and M. Kasai. 2003. *Candida tropicalis* in a neonatal intensive care unit: Epidemiologic and molecular analysis of an outbreak of infection with an uncommon neonatal pathogen. *J. Clin. Microbiol*., 41(2): 735-41.

Sain, D.O., S.S. Ginda and L.P. Ryvniak. 1991. Immunologic reactivity in patients with disseminated pulmonary tuberculosis with polysensitization. *Probl. Tuberk*., 6: 58-63.

Schell, W.A.1995. New aspect of emerging fungal pathogen. A multifaceted challenge. In: Contemporary issues in clinical microbiology. (Ed.): J.D. Christic. *Clin. Lab. Med*., 15:365-387.

Solov'eva, T.N., Z.O. Karaev, S.M. Ignateva and N. Mizonov. 1991. The diagnosis of mycotic infections in patients with respiratory tuberculosis. *Probl. Tuberk*., 7: 37-40.

Wenzel, R.P. 1995. Nosocomial Candidemia: Risk factors and attributable mortality. *Clin. Infect. Dis*., 20(6): 1531-1534.

(Received for publication 26 July 2004)