International Journal of Multidisciplinary Research and Development

IJMRD 2015; 2(2): 487-490
www.allsubjectjournal.com
Received: 04-02-2015
Accepted: 17-02-2015
E-ISSN: 2349-4182
P-ISSN: 2349-5979
Impact factor: 3.762

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Mycological spectrum in Sputum samples of Pulmonary Tuberculosis Attending TB Clinic

J Mowna, Dinesh Kaliyamoorthi

Abstract
Tuberculosis patients are in immunocompramised status, this makes the fungal infections to occur in early stage. In the present study we focus the attention on opportunistic fungal pathogens that are present in pulmonary tuberculosis, which may help in correcting the diagnosis.All the sputum specimens were subjected potassium hydroxide (KOH) preparation and Gram stain. Mycological culture was done on Sabourauds dextrose agar. Briefly, purulent portion of the sample was inoculated onto the media and incubated at 30°.Among the 152 sputum samples that were analyzed fungal elements were seen in 24/152 (15.7%), yeast cell with pseudohypae 28/152 (18.4%). Pulmonary fungal pathogens isolated in TB patients were Candida albicans 25/152 (16.4%), Candida guilliermondii 1/152 (1.52%) and Candida tropicalis 2/152 (3%), Aspergillus niger 14/152 (9%), A.fumigatus 10/152 (6%), A. flavus 6/152 (3.9%).

Keywords: Tuberculosis, Fungus, Candida, Aspergillums, Potassium hydroxide, Sabourauds Dextrose Agar.

1. Introduction
Tuberculosis in India kills about more than 1000 people a day per every minute. Tuberculosis kills more population than the other infectious disease. Opportunistic infection load will increase tremendously by 21st century due to the increase in immunocompromised capacity of the host particularly in patients with Tuberculosis, cancer, and AIDS. Tuberculosis patients usually take a prolonged treatment with antibiotics and vitamins due to the immunocompromised status, this makes the fungal infections to occur in early stage. Some patients show the symptoms of tuberculosis in spite of X-ray showing negative report, this is found to be because of the opportunistic infections found in the cavities. In the present study we focus the attention on opportunistic fungal pathogens, their infections and identification of such fungi that are present in pulmonary tuberculosis, which may help in correcting the diagnosis.

2. Objective
To study the fungus causing opportunistic infection in Tuberculosis positive patients which may be because of their underlining immunological status.

3. Materials and methods
This study was conducted between august 2014 to December 2014 in Shri Sathya Sai Medical College and Research Institute. A total of 96 sputum samples were collected from adults who were suspected to be tuberculosis positive, as defined the World Health Organization (WHO) clinical criteria of persistent cough, loss of weight and night sweats and confirmed by ZN smear. All the patients were requested to produce first morning sputum samples. The samples were subjected to mycological investigation using microscopy and culture.

4. Inclusion criteria
- All adults with smear positive Tuberculosis.

5. Exclusion criteria
- Children
- Patients with extra pulmonary tuberculosis.

6. Methods

All the sputum specimens were subjected potassium hydroxide (KOH) preparation for fungal elements. Also all the sputum sample direct Gram stain was observed to determine the budding yeast crll and pseudohyphae with pus cells. Briefly, purulent portion of the specimen was mixed with 10% KOH left to stand for 20minutes, and examined at X40 magnification for fungal elements. Mycological culture was done on Sabourauds dextrose agar. Briefly, purulent portion of the sample was inoculated onto the media and incubated at 30°C and for up to four weeks, before the culture was reported negative. Positive mould cultures were identified morphologically using macro and micromorphological features on Lactophenol cotton Blue mount.

7. Result

152 patients were included in this study, in which the gender participants include, 96/152 (63.2%) males and 56/152 (36.8%) were females [Chart - I]. Among the 152 sputum samples that were analyzed fungal elements were seen in 24/152 (15.7%), yeast cell with pseudohypae 28/152 (18.4%) [Chart – II]. Pulmonary fungal pathogens isolated in TB patients were 58/152 (38.1%), yeast with pseudohypae were isolated in 28/152 patients which included Candida albicans 25/152 (16.4%), Candida guilliermondii 1/152 (1.52%) and Candida tropicalis 2/152 (3%) was isolated [Chart – III]. Filamentous fungus associated in TB patients are as follows: Aspergillus niger 14/152 (9%), A.fumigatus 10/152 (6%), A.flavus 6/152 (3.9%). Among the number of pathogens isolated from TB patients yeast with pseudohyphae were 28/152 (18.4%) and filamentous fungi were 30/152 (19.7%), samples negative for fungal elements 94/152 (61.8%).

8. Discussion

The expanding population of the immunocompramised patients, the frequency of invasive mycosis due to opportunistic fungal pathogens had increased significantly over the past decades. The increased infection is inversely associated with increased morbidity and mortality. This is directly related to the development of serious fungal infections in the risk group patients. The patients at risk include individuals undergoing organ transplant , immunosuppressive therapy, Blood and bone marrow transplantation , premature birth, extremes of age[1].

The most common opportunistic fungal organisms causing disease in immunocompramised patients are Candida species, Aspergilus species, Mucor species and Cryptococcus neoformans [2, 3]. In a total of 152 patients included in the study 24 out of 152 (15.4%) showed fungal elements and 28/ 152 (18.4%) Yeast cells. The reason for increased prevalence are lowering of immune system due to tuberculosis and the prolonged use of anti tuberculous drugs which promote the growth and reproduction of the fungal flora and in turn , Candida albicans is the most common fungal co-infection with Mycobacterium tuberculosis , with a prevalence of 25/152 (16.4%). These opportunistic fungi are potent pathogens in the aggravate the course of underline process in the lung tissues [4]. Our study showed that immunocompramised individuals, patients with some pre-existing disease and patients with long history of antibiotic usage. [2, 5].Mycobacterium tuberculosis has been found to enhance the growth of C.albicans on Lowenstien - Jensen media. Studies have confirmed that polysaccharide fraction of C,albicans enhances the growth and reduction of the generation time of Tubercle bacilli . [6]. this indicates the high prevalence of C. Albicans association with tuberculosis patients and C, albicans is also recognised as the most common opportunistic pathogen in HIV/AIDS by the WHO. [2].

All the samples were obtained from adults. Among them males showed high incidence 96/152(63.2%) and females were 56/152(36.8%). This is because of the health seeking behaviour of males which make them at risk of TB infection than females. [7]. Aspergillus is one of the most common fungus to cause the disease in immunocompramised individuals .In recent years it hsa been shown that Aspergillus infection can result in a broad range of air way complications with radiological and pathological picture mimicking those pf TB. [8]. Aspergillus Niger was the most common fungal mold isolated in our study 14/152(9%) next to Aspergillus fumigates 10/152(6%) followed by Aspergillus flavus 6/152(3.9%). Aspergillus niger is less likely to cause human diseases when compared to other aspergillus species, but if large amount of spores are inhaled a serious lung disease, Aspergillosis can occur.

Chronic obstructive pulmonary disease due to coexistence of Mycobaterium and Aspergillus is a fatal combination leading to progressive lung destruction, resulting in early death despite prolonged anti mycobacterial chemotherapy [9-12].

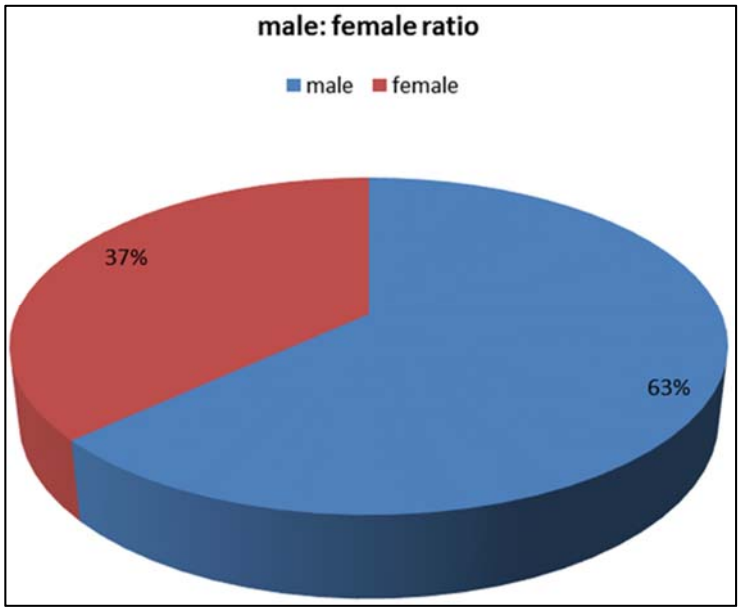

Chart: 1

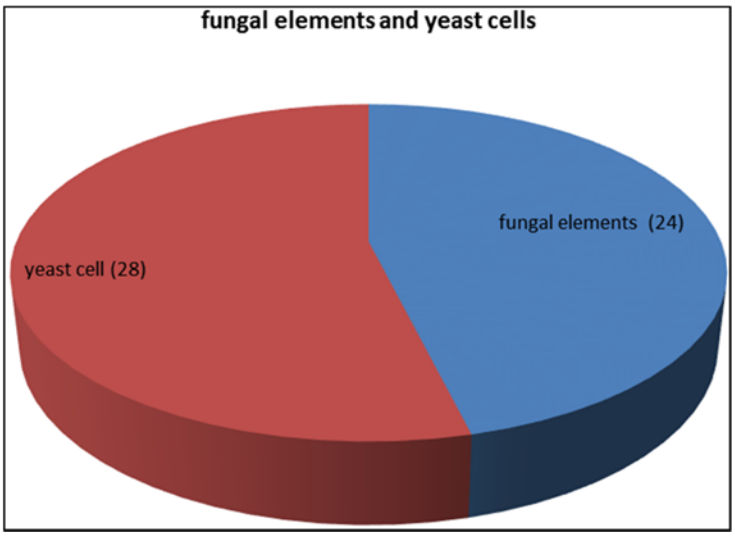

Chart: 2

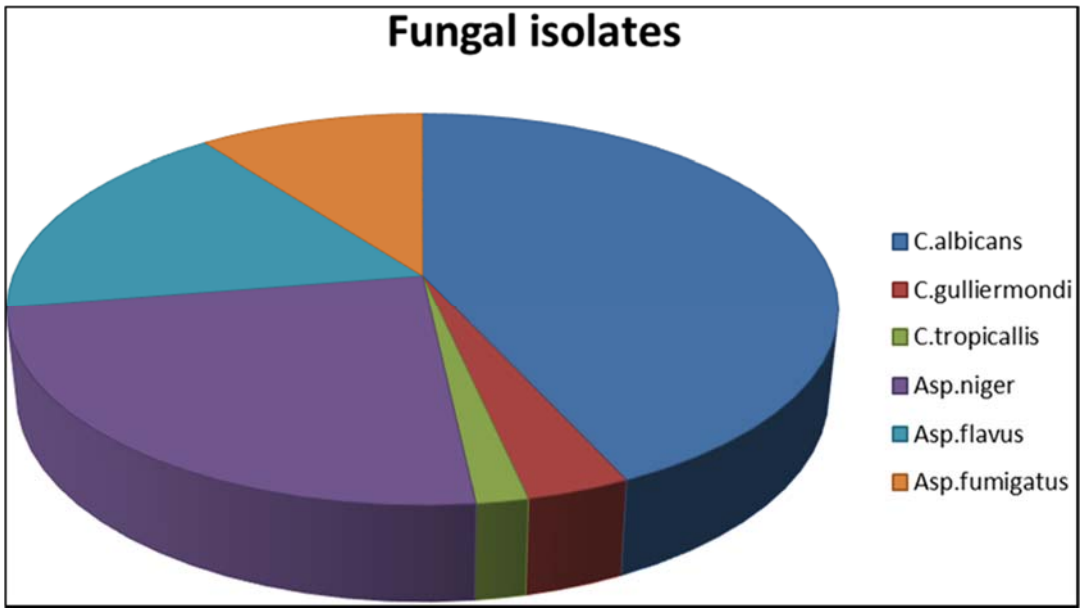

Chart: 3

Male Female ratio / Fungus Isolated	Male(96)	Male %	Female(56)	Female %
Candida albicans	17	17.7	8	7.68
Candida gulliermondi	2	1.92	0	0
Candida tropicalis	1	0.96	0	0
Aspergillus niger	8	7.68	6	5.76
Aspergillus flavus	6	5.76	4	3.84
Aspergillus fumigatus	4	3.84	2	1.92
Total	38	36.48	20	19.2

Table 1:

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