Prevalence of Opportunistic Fungal Infection in Pulmonary Tuberculous Patients

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Abstract

Background: Pulmonary fungal infections have emerged as a world-wide health care problem in recent years**.** Pulmonary TB is one of the most common predisposing factors. Chronic nature of tubercular infection along with prolonged chemotherapy with or without corticosteroids severely affects patient's immune system predisposing him to super infection by opportunistic fungi. **Objective:** The current study includes 100 selected adult patients who presented with persistent, multidrug resistant or relapsed pulmonary TB. Early morning sputum was collected from each case. The sputum was expectorated from lower respiratory tract and collected in a sterile container. The samples were inoculated onto sabouraud's dextrose agar with chloramphenical (50 mg/ml) and incubated at 25 ^{0}C for 6 weeks. Then the growth form**,** rate of growth, surface and reversed coloration on SDA plates were noted. **Results**: 65% patients were males. Age was ranging from 22 to 65 years, commonest age of presentation was 31-40 years and the mean age ± SD was (40.1 ± 10.8) years. Fungal species were isolated in 47 samples (47%); the most common isolated fungi were *Aspergillus species* (55.3%). Maximum positive culture results (42.5%) were obtained in the age group from 31 to 40 years. Positive fungal culture was significantly associated with male gender, smoking and DM as the P values were 0.02, 0.02 and 0.0006 respectively. **Conclusion:** Chronic pulmonary infection carries the risk of higher percentage of opportunistic fungal infections. *Aspergillus* was the predominant species. Male gender, smoking and diabetes are considered as risk factors that increase the chance of fungal infection.

Keywords: Pulmonary fungal infection, fungal infection in tuberculosis.

الخلاصة

المقدمة: اصبح الالتهاب الفطري الرئوي يمثل مشكلة رعاية صحية في جميع انحاء العالم في السنوات الاخيرة. يعتبر السل الرئوي العامل الاكثر شيوعاً بين العوامل المهيئة لهذا النوع من الالتهابات. ان طبيعة الالتهاب المزمنة للسل الرئوي اضافة الى استخدام العلاج الكيمياوي مع او بدون استخدام الستيرويدات يؤثر بشدة على وظيفة الجهاز المناعي للمريض ويجعله عرضة للاصابة بالالتهاب الفطري الانتهازي. **المواد والطرائق:** تضمنت الدراسة الحالية 100 مريض من البالغين والذين كانوا يعانون من سل رئوي مستمر, سل رئوي مقاوم للعديد من الادوية او سل رئوي انتكاسي. تم تجميع بلغم من كل مريض في الصباح الباكر. تم تقشيع البلغم من المجاري التنفسية السفلى وتجميعه في حافظة معقمة. تم تطعيم عينات البلغم المجموعة على أغار دكستروز سابورو مع كلورومفينيكول (50 ملغم/ملليتر) وتحضينها بدرجة حرارة 25م^{5} لمدة ستة اسابيع, ثم تم دراسة وملاحظة اشكال النمو, معدل النمو, وتغير سطح ولون اللوح المزروع. **النتائج:** 65% من المرضى كانوا ذكور. كانت اعمار المرضى تتراوح بين 22− 65 سنة, اكبر نسبة للاعمار تراوحت بين 31− 40 سنة وكان معدل الاعمار ± الانحراف المعياري (40.1 ± 10.8 سنة). ظهرت نتيجة زرع الفطريات موجبة في 47% من العينات وكان اكثر انواع الفطريات المسببة للالتهاب هو *Aspergillus species* بنسبة 55.3%. اعلى نسبة من العينات الموجبة (42.5%) ظهرت في المرضى الذين تتراوح اعمارهم بين 31− 40 سنة. كانت نتائج العينات الموجبة مرتبطة بشكل كبير بالجنس الذكري, التدخين وداء السكري حيث كانت قيمة **P** 0.02, 0.02 و 0.006 على التوالي. **الاستنتاج:** تشتمل الالتهابات الرئوية المزمنة على خطورة كبيرة لحدوث الالتهابات الفطرية الانتهازية. اكثر انواع الفطريات المسببة لهذا النوع من الالتهاب هي الاسبرجلاس. يعتبر الجنس الذكري, التدخين وداء السكري اكثر عوامل الخطرالتي تزيد نسبة الاصابة بهذا الالتهاب.

الكلمات المفتاحية: التهاب فطري رئوي, التهاب فطري في السل الرئوي.

Introduction

Pulmonary fungal infections have emerged as a world-wide health care problem in recent years, owing to the extensive use of broad-spectrum antibiotics, long-term use of immunosuppressive agents, and the increasing population of terminally ill, debilitated and immunocompromised patients such as those with pulmonary tuberculosis (TB), diabetes mellitus or malignancy (Taura *et al.,* 2014). It can be acquired primarily or secondarily in immunosuppressive conditions and may worsen the primary disease. Pulmonary TB is one of the most common predisposing factors for development of pulmonary fungal infection (Osman *et al.,* 2013). Chronic nature of tubercular infection along with prolonged chemotherapy with or without corticosteroids severely affects patient's immune system predisposing it to super infection by opportunistic fungi. Greer and Gemoets suggested a tendency for pulmonary tuberculosis to develop a more devastating course when it is associated with an invasion of parasitic fungi thus increasing the activity and virulence of tuberculosis (Yadu *et al.,* 2015). Mainly four types of fungi; *Aspergillus niger*, *A. fumigatus, Histoplasma capsulatum* and *Cryptococcus neoformans* were recorded, which cause severe infection in lungs in patients suffering from pulmonary tuberculosis (Osman *et al.,* 2013). *Aspergillus* is an airborne fungus that everyone breathes in daily. It does not normally cause illness, except in people with a weakened immune system. The symptoms of chronic pulmonary aspergillosis – weight loss, severe shortness of breath, fatigue and coughing up blood – are so similar to those of TB that physicians often misdiagnose and prescribe the wrong treatment. The infection can grow undetected for years, by which time it is too late to treat successfully. These opportunistic infections if diagnosed early can be treated effectively to prevent the prognosis of disease (Denning and Cole, 2011). Building on knowledge accrued in the last several decades regarding the cell wall composition of *Aspergillus* and the endemic fungi, *Histoplasma capsulatum* and *Cryptococcus neoformans*, diagnostic tests for detecting these fungi have become a routine part of the approach to diagnosis. Antigen detection can be accomplished in urine, serum and body fluids, including respiratory samples obtained by bronchoalveolar lavage (BAL). Especially in immunocompromised patients, in whom antibody responses are notoriously poor; antigen detection has led to earlier diagnosis and improved outcomes (Smith and Kauffman, 2012).

The aim of the study is to investigate the prevalence of fungal infection, the most common fungal species and possible risk factors predisposing to opportunistic mycosis in patients with pulmonary tuberculosis.

Materials And Methods

1. Patients:

The study included 100 selected patients that diagnosed with pulmonary TB who attended Pulmonary TB Institute in Baghdad from February 2012 to January 2014. Patients specimen included adults of both genders who presented with **persistent TB**: defined as a persistence of symptoms despite full course of chemotherapy, **multidrug resistant TB**: defined as resistance to any combination of anti-TB drugs that include INH and rifampicin or **relapsed TB**: defined as a patient who has been declared cured of any form of TB in the past by a physician after one full course of chemotherapy, and has become sputum smear positive pulmonary TB (Osman et al. 2013), while cured cases were excluded. Informed consent was obtained from each patient prior to specimen collection.

2. **Sputum collection:**

Early morning sputum was collected from each case and transported to the laboratory. Sputum samples from patients were collected with the assistance of experienced medical laboratory workers. The sputum was expectorated from lower respiratory tract and collected in a sterile dry wide-necked, leak-proof container to avoid contamination from external sources (Taura et al. 2014). Patients were asked to produce the samples in an open air space away from other people to avoid aerosol spread. The patients were instructed to inhale deeply 3 to 4 times before coughing out from the chest. The sputum produced was carefully spit into the container without contaminating the outside of the container. The lid of the container was screwed tightly before being processed, with utmost care not wrapping the container (Yahaya et al. 2015).

3. **Fungus culture**:

Further process was done such as direct examination and culture. Direct examination of specimens was done by:

I. Direct KOH Mount (10% Potassium Hydroxide)

II. Gram's Stain

Then samples were inoculated onto sabouraud's dextrose agar (SDA) with chloramphenical (50 mg/ml) and incubated at 25 ^{0}C and observed at regular intervals for 6 weeks (Babita et al. 2016). After appropriate incubation, the growth form**,** rate of growth, surface and reversed coloration on SDA plates were noted. The yeast like colonies were identified using Biochemical and Physiological tests viz. Germtube production, chlamydospore formation on cornmeal agar, sugar fermentation and assimilation tests. Pure isolates were obtained by sub culturing on new plates and colonies growing out of the inoculation areas were regarded as contaminants. Moulds isolated were identified with their distinctive morphological features associated with the characteristic sporing heads, septa formation…etc (Yadu et al. 2015).

Results and Discussion

Fungal infections remain a leading cause of infectious morbidity and mortality in heavily immunosuppressed patients (Sahoo, 2006). Although active mycosis may be an independent marker of advanced immunosuppresion, it may also act as co–factor in accelerating and amplifying the clinical course of tuberculosis disease (Whalcn *et al.,* 2000). Early initiation of effective antifungal therapy and reversal of underlying host defects remain the cornerstones of treatment for pulmonary fungal infections (Richardson and Florl, 2008).

Majority of our patients were males (65%) as shown in figure 1. Men are more vulnerable to infection than females due to their greater exposure to the surrounding. Other studies showed similar results, as the percentage of male patients were 72%, 64%, 62.5% and 58% in (Osman et al. 2013, Yadu et al. 2015, Bansod and Rai 2008 and Taura et al. 2014 respectively).

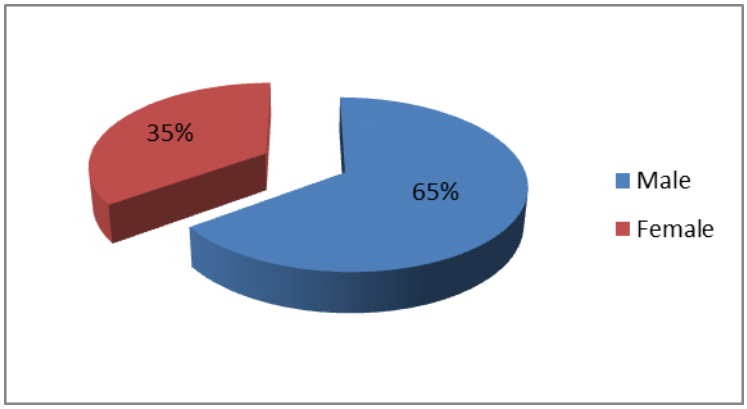

Fig. 1: gender distribution of the studied group.

Patients age was ranging from 22 to 65 years, the most common age of presentation was 31-40 years and the mean age ± SD was (40.1 ± 10.8) years as shown in figure 2. In a study done by (Bansod and Rai 2008), the maximum patients were recorded between the ages of 35 to 45 years and their percent was 36.7%.

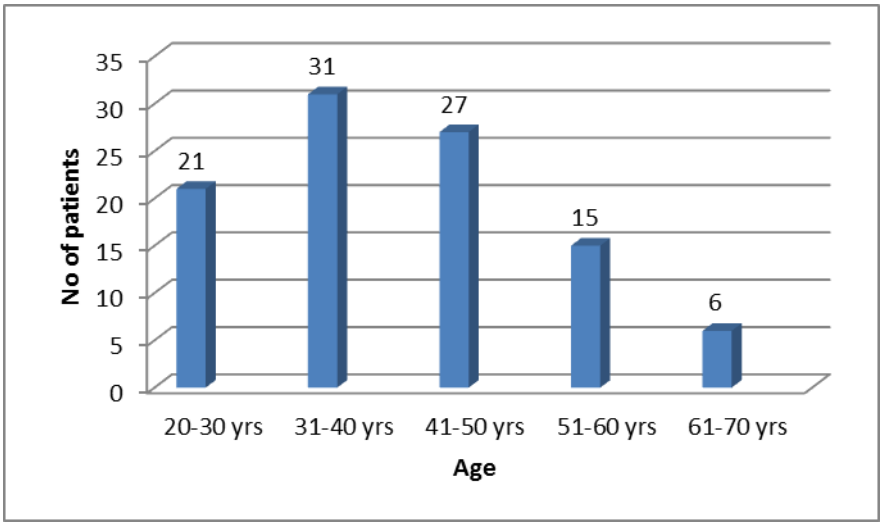

Fig. 2: age distribution of the studied group.

Fungal species were isolated in 47 samples (47%). The percentage of positive culture in other studies were 55.5%, 54.4%, 49% and 46% in studies done by (Taura et al. 2014, Gupta et al. 2012, Yadu et al. 2015 and Bansod and Rai 2008 respectively). In contrast to results obtained by (Osman *et al.*, 2013 and Shome *et al.*, 1976) as the positive cultures in these studies were 24% and 18% respectively.

The most common isolated fungi were *Aspergillus species* in 26 (55.3%) samples, among them *Aspergillus fumigates* was the most common (31.9%) and the distribution of different types of isolated fungi is shown in table 1. In other studies performed by (Chen *et al.*, 2001, Babita *et al.*, 2016, Ekenna *et al.*, 2007, Taura *et al.*, 2014, Kurhade *et al.*, 2002 and Osman *et al.*, 2013), *Aspergillus species* were also the predominant fungi detected in the following percentages 57%, 55.6%, 42.9, 36.9% and 24% respectively. While (Yadu *et al.*, 2015, Anna *et al.*, 2012 and Khanna *et al.*, 1977) found that *candida* were the dominant fungi in the following percentages 34.69%, 27.5% and 26.36% respectively.

Table 1: Percentages of isolated fungi

Fungal species	No of patients	percentage
Aspergillus fumigatus	15	31.9%
Aspergillus niger	11	23.4%
Candida albicans	8	17.1%
Cryptococcus neoformans	7	14.9%
Histoplasma capsulatum	6	12.7%
Total number	47	100

Positive fungal culture was significantly found in 36 (55.4%) of the male patients compared to 11 (31.4%) of the females as the P value was 0.02 (table 2). Bansod and Rai 2008 and Osman et al. 2013 also found significant association of positive culture with male gender. While Chen et al. 2001 and Hidalgo and Vazquez 2004 found that no significant difference in gender ratio was demonstrated.

Table 2: Response of fungal pathogens according to the gender of patients

Gender	No.	+ve	%	-ve	%	P value
Male	65	36	55.4	29	44.6	0.02
Female	35	11	31.4	24	68.6	
Total	100	47	---	53	---	

Maximum positive culture results (42.5%) were obtained in the age group from 31 to 40 years (table 3). Taura et al. 2014 found that the age group 31-40 was found to have the highest incidence (27.92%). Yadu *et al.*, 2015 found a mean age of 38.03 years amongst male and 30.82 years amongst females were culture positive for fungal agents. Yahaya *et al.*, 2015 found that the age is dependant of these mycotic infections as lower ages below 10 years did not show the presence of the systemic infection and old ages greater than 76 years also show very low prevalence with middle age groups showing high prevalence possibly due to high environmental exposure of these age groups, particularly in secondary infections. While Osman *et al.*, 2013 and Chen *et al.*, 2001 did not find any significant relation between culture results and age.

Table 3: Response of fungal pathogens according to the age of patients

Age (yrs)	20-30	31-40	41-50	51-60	61-70	Total
No.	7	20	9	8	3	47
%	14.9	42.5	19.2	17	6.4	100

Sixty three patients were smokers, positive fungal culture was significantly found in 35 (55.6%) of smokers compared to 12 (32.4%) of non smokers as the P value was 0.02 (table 4). Osman *et al.*, 2013 agreed with this result while Taura *et al.*, 2014 did not find any significant association.

Table 4: Response of fungal pathogens according to smoking status of patients

Smoking status	No.	+ve	%	-ve	%	P value
Smoker	63	35	55.6	28	44.4	0.02
Non smoker	37	12	32.4	25	67.6	

Total	100	47	---	53	---	

Thirty two patients were diabetics, positive fungal culture was significantly found in 23 (55.6%) of diabetics compared to 10 (32.4%) of non diabetics as the P value was 0.0006 (table 5). Same finding was obtained by Osman *et al.*, 2013, Ekenna *et al.*, 2007 and Jain 1982.

Table 5: Response of fungal pathogens according to diabetic status of patients

Diabetes status	No.	+ve	%	-ve	%	P value
Diabetics	32	23	71.9	9	28.1	0.0006
Non diabetics	68	24	35.3	44	64.7	
Total	100	47	---	53	---	

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