Sys Rev Pharm 2021;12(1):1393-1396
A multifaceted review journal in the field of pharmacy

Prevalence Of Fungi In Clinically Suspected Cases Of Pulmonary Tuberculosis In Iraq, Wasit

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ABSTRACT

Pulmonary mycosis may be due to by either opportunistic or endemic fungi or a combination of the two. The death rate in pulmonary fungal infection may be more than 90% in patients with immune-compromised although patients who are immunocompetent respond well to treatment. Diagnosis of pulmonary disease caused by fungi considered challenge, because pulmonary mycosis may like other diseases (for example lung cancer, viral, bacterial pneumonia or pulmonary tuberculosis). During work in medical laboratories, repeated elements of fungi were observed in sputum smears of acid-fast bacilli taken from patients suspected having pulmonary tuberculosis, which required investigating of the occurrence of pulmonary mycosis in these cases. A total of 158 sputum samples were collected from clinically suspected pulmonary tuberculosis patients. Two sputum samples were taken in the early morning during two days. Microscopic examination of sputum samples revealed 20 positive result. Five samples failed to be detected; However later showed a positive result in culture The common fungi isolated were Candida species 15 (9.5 %) followed by Aspergillus species 10 (6.3 %). The isolated Candida species were C. albicans 8 (5.1%), C. tropicalis 5 (3.2%), C. krusei 2 (1.3%) whereas the Aspergillus species were comprised of A. fumigatus 5 (3.2%), A. flavus 3 (1.9%) and A. niger 2(1.3%).

Keywords: Pulmonary mycosis, Tuberculosis, Culture.

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INTRODUCTION

Pulmonary infections are among the most common conditions in inpatient and outpatient clinical care. Fungal infections are an increasingly frequent cause of pulmonary disease worldwide [1]. May be as result of the increasing population of immunocompromised hosts resulting from chemotherapy, organ transplantation, AIDS and chronic steroid use. Both lymphocytic deficiencies and neutropenia predispose to mycotic infection[2].

Fungal infection of the lungs also called pulmonary mycosis which may be caused by either opportunistic or endemic fungi or a combination of the two. The death rate in pulmonary mycosis may be more than 90% in patients with immune-compromised although patients who are immunocompetent respond well to treatment [3,4].Pulmonary fungal infection in humans can occur due to inhalation of fungal spores, hematogenous dissemination or reactivation of a latent infection. Immunocompromised children, as well as those from certain regions where endemic fungal infections occur are at highest risk[5].The clinical presentation can various from asymptomatic to disseminated fatal disease, depends on the status of immune and the dose of infectious from the environmental exposure. Clinical symptoms are nonspecific and include cough, fatigue, dyspnea and may be associated with hemoptysis[6]. Diagnosis of pulmonary illness caused by fungi considered challenge, because pulmonary fungal infections may like various diseases (for example lung cancer, viral, bacterial pneumonia or pulmonary tuberculosis); Doctors in low--pervasiveness settings may not be acquainted with the illness manifestations. Building up a diagnosis is additionally convoluted by the trouble in developing these micro-organisms and by the scarcity of non-culture -based demonstrative examines, specifically the absence of standardization of serological and molecular tests. Pathologists may not be acquainted with the histopathological features[7].

During work in medical laboratories, repeated elements of fungi were observed in sputum smears of acid-fast bacilli taken from patients suspected having pulmonary tuberculosis. Moreover, in Wasit, Iraq, little or no work was done on pulmonary fungal infection, this prompted us to investigation about the prevalence of pulmonary fungal infections in these cases that were admitted to AL-Zahraa teaching hospital and other centers in this city. Therefore, the objectives of the study were to find out the prevalence of fungal infections in patients suspected having pulmonary tuberculosis and to identify common fungi in these cases.

MATERIAL AND METHODS:

One hundred fifty-eight sputum samples were collected from patients with clinically suspected pulmonary tuberculosis attending medical laboratory-unit of microbiology. A prospective cross sectional study was conducted in AL-Zahraa teaching hospital and other centers in this city after obtaining informed consent from each patient prior to specimen collection. The study was carried out from October 2018 to July 2019.

Specimen collection

Two sputum samples were taken in the early morning during two days and collected in sterile containers .None of patients was undergoing anti-fungal treatment.

Specimens processing

A. microscopically examination

Ziehl-Neelsen method of acid-fast staining: Sputum samples were stained depending on Acid Fast procedure;

Stained slides were examine under oil immersion objective for detection of acid fast bacilli if present.

Potassium hydroxide (KOH) mount: large drop of KOH (10-20)% was placed in the center of a clean slide, a small portion of sputum transferred by using sterile wire loop into the KOH drop then mixed well. The slide was placed in a moist chamber at room temperature and kept for about 30 minute, then examined under low power for detection of fungi.

Lactophenol Cotton blue staining method: Lactophenol Cotton blue (LPCB) staining method considered one of the most useful identification techniques used in the preparation of slides for microscopic examination of fungi (hyphae or yeast). Fungal elements are stained intensely blue.

Fungal culture

All samples were cultured on slant tube of Sabouraud's Dextrose Agar (SDA), containing Gentamycin 20 mg/L, chloramphenicol 50 mg/L and tetracycline 50 mg/L, incubated at 30°C-35°C and examined daily to observe the growth. Samples were declared as negative only if there was no growth after six weeks of incubation. The isolated fungi were identified depending on their macroscopic and microscopic features by Lactophenol cotton blue smear.

Statistical analyses.

Data entered and managed by using statistical package for social sciences (SPSS) Version 20 for windows. P-value of < 0.05 considered statistically significant.

RESULTS

Fungal infections were observed in 25 sputum samples out of 158 patients clinically suspected pulmonary tuberculosis. Fungi were detected as a primary etiological agents in 23 (92.0%) and 2 (8.0%) as a secondary etiological agents; Whereas 28 were positive only for AFB, as shown in Table (1&2).

Table 1: Association of fungal culture with demography of study group

Factors		Results		*P*. value
		Positive N= 25	Negative N= 133	
Sex	Male	11 (44.0%)	88 (66.2%)	0.036
	Female	14 (56.0%)	45 (33.8%)	
Age (mean) 41.23. Range (17 – 63)		40.92 (22 – 63)	41.29 (17 – 62)	0.886
Smoking	Yes	11 (44.0%)	36 (27.1%)	0.089
	No	14 (56.0%)	97 (72.9%)	
Drinking	Yes	1 (4.0%)	10 (7.5%)	0.526
	No	24 (96.0%)	123 (92.5%)	
Chronic status	DM	11 (44.0%)	11 (8.3%)	0.00
	SLE	2 (8.0%)	0 (0.0%)	
	Leukemia	1 (4.0%)	0 (0.0%)	
	No	11 (44.0%)	122 (91.7%)	

Table 2: Fungi causing primary and secondary pulmonary infection

Fungus	Primary pulmonary infection (%)	Secondary pulmonary infection (%)	Total (%)
Candida albicans	7 (4.4%)	1 (0.6%)	8 (5%)
Candida tropicalis	4 (2.5%)	1(0.6%)	5 (3.1%)
Candida krusei	2 (1.3%)	0 (0.0%)	2 (1.3%)
Total *Candida* species	13 (8.2%)	2 (1.3%)	15 (9.5%)
Aspergillus fumigatus	5 (3.2)	0 (0.0%)	5 (3.2%)
Aspergillus flavus	3 (1.9%)	0 (0.0%)	3 (1.9%)
Aspergillus niger	2 (1.3%)	0 (0.0%)	2 (1.3%)
Total *Aspergillus* species	10 (6.32%)	0 (0.0%)	10 (6.32%)

The common fungi isolated were *Candida species* 15 (9.5 %) followed by *Aspergillus species* 10 (6.3 %). The isolated *Candida* species were as the following: *Candida albicans* 8 (5.1%), *Candida tropicalis* 5 (3.2%), *Candida krusei* 2 (1.3%) whereas species of *Aspergillus* including: *Aspergillus fumigatus* 5 (3.2%), *Aspergillus flavus* 3 (1.9%) and *Aspergillus niger* 2(1.3%), as shown in Table 3.

Table 3: Frequency of pulmonary fungal infection

Fungus species	Frequency	Percent %	Total	Percent of infection %
Negative	133	84.2	84.2	
Candida albicans	8	5.1	9.5	32
Candida tropicalis	5	3.2		20
Candida krusei	2	1.3		8
Aspergillus fumigatus	5	3.2	6.3	20
Aspergillus flavus	3	1.9		12
Aspergillus niger	2	1.3		8
Total	158	100%	100%	100%

Table 4: Frequency distribution of TB with results of fungal culture

		Fungal Culture		Total	P. value
		Negative	Positive		
TB	**Positive**	28 (21.1%)	2 (8.0%)	30 (19.0%)	0.127
	Negative	105 (78.9%)	23 (92.0%)	128 (81.0%)	
Total		133 (100.0%)	25 (100.0%)	158 (100.0%)	

Microscopic examination of 158 sputum samples collected from clinically suspected pulmonary tuberculosis cases revealed twenty positive result. Five samples failed to be detected microscopically, but later showed a positive result in culture, as shown in Table 5.

Table 5: Evaluation of direct microscopic examination comparison with gold standard test (culture)

Laboratory techniques		Culture		Sensitivity	Specificity	PPV	NPV
		Negative (No.)%	Positive (No.)%				
Direct examination (KOH)	Positive	0 (0.0%)	20 (80.0%)	80%	100%	100%	96%
	Negative	133 (100.0%)	5 (20.0%)				
Direct examination (LPCP)	Positive	0 (0.0%)	20 (80.0%)	80%	100%	100%	96%
	Negative	133 (100.0%)	5 (20.0%)				

DISCUSSION

Pulmonary mycoses in certain regions of the world cause high rates of morbidity and mortality, which may be present in healthy people and can cause disease when the host immunity is defective or low. Diagnosis and management remain challenging due to the lack of pathognomonic and radiological features and especially in low-prevalence regions, where there are low awareness of pulmonary mycosis association with the absence of mycology laboratory tests. Fortunately, the laboratory procedures adopted by the current study were successful in detecting the fungal infections of the cases who those suspected to have pulmonary tuberculosis. Although treatment is difficult, today it is important to know these infections well and manage them scientifically. These diseases, if diagnosed early can be effectively treated and prevent progression it.

Prevalence of pulmonary mycosis

Of the total cases covered by the current study, fungal infections were diagnosed in 25 (15.8 %) cases. The study demonstrated that *Candida* species 15 (9.5%) and *Aspergillus* species 10 (6.3%) are the main causes of pulmonary mycoses, amongst yeasts, *C. albicans* was predominant species 8 (5.1%) following by *C. tropicalis* 5 (3.2 %) and *C. krusei* 2 (1.3%), Whereas *Aspergillus* species included *A. fumigatus* as the most common species 5 (3.2%) followed by *A. flavus* 3 (1.9%) and *A. niger* 2 (1.3%) and these findings agreement with study done by Chalana M, *et al*.(2019)[8]. Infected males were 11 (44.0 %) and females were 14 (56.0%) out of all patients with Pulmonary mycosis , there was significant statistical difference between males and females .The result of the current study was consistent with those reported by Buthia T, Adhikari L (2015); Soedarsono S, *et al*.(2020)[9,10]. There was no statistical significance associated between age and prevalence of pulmonary fungal pathogens (P = 0.886) agreement with what was reported in study of Sani FM, *et al*.(2020)[11].

The study proved that direct examination of samples is recommended to establish possible fungal infection and treatment with antifungal agents. However, due to limited sensitivity, a negative direct examination should be not exclude infection; Which was similar to previous published study in India[8]. Secondary pulmonary mycosis 2 (8.0%) was significantly less common compared to Primary pulmonary mycosis 23 (92.0 %).This result agreement with a study done by Sani FM, *et al.* (2020)[11].

The reason for the present of secondary mycosis may be due to immunosuppression as result of tuberculosis and also, the prolonged use of antibiotics without consulting specialized clinics, which promote the overgrowth of the fungus. In the current study chronic status (included SLE, diabetes and leukemia) were important and major risk factor for pulmonary mycosis; And this came in consistent with what was mentioned in the study done by Firacative C, *et al*.(2020)[12]. Smoking was statistically less significant (P = 0.089) in comparison with what was reported in the study of Sani FM, *et al*.(2020)[11].

CONCLUSION

Fungal infections should be considered when investigating tuberculosis. The study also showed the importance of relying on fungal culture in association with microscopic examination.

ACKNOWLEDGMENT

Grateful to all members of Medical Laboratory Department, AL-Zahraa Teaching Hospital in Wasit city for their help and cooperation.

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