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Pulmonary Mycosis in Chronic Obstructive Airway Diseases and Effects of Some Medicinal Plant Extracts

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Abstract

Pulmonary mycosis is not uncommon finding in patients complaining of chronic obstructive airway diseases. Excess use of corticosteroids enhances the infection. Canadida and Aspergillus species were the predominant strains isolated. The isolated fungi were sensitive (in vitro) in different degrees to different medicinal plant extracts.

Introduction

FUNGI are abundant in the environment. They live in the soil as saprophytes, deriving nutrients from dead plants and animal matter. Their spores are produced in great abundance and are readily disseminated into air by wind currents[**1**].

The incidence of fungal respiratory tract infections is increasing, this could be explained by the increased awareness and consequent search for fungal infection by appropriate techniques[**2,3,4**] and also due to promotion of fungal growth by abusing broad spectrum antibiotics and excess use of corticosteroids and other immunosuppressive drugs[**5**]. Many investigators isolated different kinds of fungi in different respiratory diseases[**6,2,4**].

Inhalant spores are a major source for respiratory tract infection. This may lead to asymptomatic or mild pulmonary infection that heals spontaneously, progressive pulmonary infection or spread to extra-pulmonary sites that occur, less frequently[**7**].

Chronic obstructive airway diseases including bronchial asthma, promote infection with fungi, a variety of both saprophytic and opportunistic fungi could be involved. Fungistatic action and fungicidal effect of medicinal plants is well-documented[**8,9,10**].

The aim of the present work is to study the most prevalent fungi in those patients, its relation to the degree of bronchial obstruction, and both the fungistatic and fungicidal effects of medicinal plants on isolated fungi.

Material and Methods

The present work included 87 patients of both sexes and different age groups that ranged from 6 to 53 years old.

These patients presented to outpatients clinic of Minia University Hospital and complained of respiratory tract infection. A thorough history, clinical examination, chest x-ray as well as complete blood picture were done for all of them.

Patients were classified into three groups :

Group I : Included 10 patients, all of them were using bronchodilators and were under oral corticosteroids, dose ranging from 5 - 10 mg prednisolone/day, duration of therapy ranged from few months to 5 years. Antibiotics were not used.

Group II : Included 43 patients, all of them under bronchodilator drugs only.

Patients in both groups presented to the hospital because of recurrent excacerbation of symptoms (Cough, dyspnea, increased expectoration, with or without fever).

Group III : (Control) : Included 34 patients with acute tracheobronchitis.

For estimation of fungi a mouth swab and a sputum sample were collected from each patient, early in the morning and after thorough mouth wash. These collected samples were subjected for isolation and identification of fungi by culturing on Sabouraud's dextrose agar media [**10**]. This medium is composed of 40 gm dextrose, 20 gm agar, 10 gm peptone and distilled water to make one liter. After autoclaving and cooling of the medium, antibiotic combination were added to inhibit bacterial growth in the isolation plates. The antibiotic combination used was composed of 20 I.U./ml. penicillin, 40 gm/ml of dihydroxstreptomycin and 0.05% cycloheximide (Actidione). The first two antibiotics were dissolved separately in sterile distilled water while the third was dissolved in methanol prior to addition to the agar medium. The medium was then distributed aseptically in sterile Petri dishes streaking the agar with the collected cotton swabs and incubated at 30°C for two weeks. The growing colonies were identified using the different identification keys and refrences.

Test of antifungal activity of some plants :

In order to test the effect of water extracts of some medicinal plants (Zingber officinale, Cuminum cyminum, Cinnamomum Cassia, Allium and Allium cepa) on growth of some fungi isolated from studied patients (Aspergillus fumigatus, A.niger, A.flavus, Candida sp., Cladosporium Sp., Penicillium rubrum. P.chrysogenum and Fusarium oxysporum). The plant extracts were obtained by soaking 5 gms of pieces of plant in 100 ml of the distilled water for 24 hour. Plants were obtained from local market, sterilization of the extracts was concluded by passing through a 0.22 μ m pore size millipore filter. The antifungal effect of the selected plants was determined by Kirby Bauer filter paper disc method [**22**]. Spores of

the tested fungi were spread on the surface of Sabouraud dextrose agar medium[11]. Filter paper discs (0.6 cm diameter) saturated with each of the plant extract were gently applied onto the agar using sterilized forceps. All plates were incublted at 28°C for 8 days, then the inhibition zones were measured to the nearst millimeter.

The results were expressed using the following arbitrary scale :

up to 0 cm : -ve.

up to 1 cm : slight activity +,

up to 2 cm : medium activity ++,

up to 3 cm : marked activity +++,

more than 3 cm : high activity ++++.

Results

The present work included 53 asthmatics and 34 cases with acute tracheobronchitis as control. All studied cases were classified into three groups :

Group I : which included 10 asthmatics under bronchodilator therapy and steroids, three of them were males and 7 patients were females.

Group II : included 43 asthmatics under bronchodilators only, 19 males and 24 females (table 1). 22 out of 53 asthmatics were under the age of 15 years, 14 above 45 years, and the remainder were between 16 to 45 years, table (1).

Group III : (Control) : Included 34 patients with acute tracheobronchitis and comparable in age and sex, table (1).

Table (1) : Age and Sex Distribution of 87 Studied Cases.

	Age & Sex								
	0 - 15 ys.		16 - 30 ys.		31 - 45 ys.		> 45 ys.		Total
	M	F	M	F	M	F	M	F	(M & F)
Group I Bronchodilator with steroids	—	2	—	1	1	—	2	4	(10)
Group II Bronchodilator without steroids	9	11	3	4	2	6	5	3	(43)
Group III (control) Acute tracheobronchitis	4	3	2	4	4	5	5	7	(34)
Total cases	13	16	5	9	7	11	12	14	(87)
Total (M & F)	29		14		18		26		87

Table (2) : Results of Mouth Swabs and Sputum Cultures for Fungi in 87 Studied Cases.

Patients (87)	Mouth swabs				Total	Sputum				Total
	—ve		+ve			—ve		+ve		
	No.	%	No.	%		No.	%	No.	%	
Group I										
Bronchodilator with steroids	2	20	8	80	(10)	1		9	90	(10)
Group II										
Bronchodilator without steroids	24	56	19	44	(43)	29	67	14	33	(43)
Total asthmatics	26	49	27	51	(53)	30	57	23	43	(53)
Group III										
Acute tracheobronch.	28	82	6*	18	(34)	—	—	—	—	(34)

* 4 : Isolated fungi were Candida species.

* 1 : was Cladosporium sp.

* 1 : was Penicillum rubrum.

Fungi were isolated from 8 out of 10 (80%) mouth swabs, and in 90% of sputum specimens from asthmatics under corticosteroid therapy (group I). In group II, fungi were isolated from 44% & 33% of mouth swabs and sputum specimens respectively. Candida and Asprgillus species were the most common fungi isolated from asthmatic patients 11 and 10 out of 27 isolates respectively, (table 3).

In control group, fungi were isolated only from 18% of mouth swabs. Candida species were the most common isolates in this groups, table (2).

Table (4) showed the effect of some medicinal plant extracts on growth of fungi isolated from studied cases. Tested fungi were more sensitive to Allium sativum and A.cepa, less sensitive to Cinnamoum cassia. Zingber officinale, and Cuminum cyminum.

Table (3) : Number, Percentage of Occurrence (%), and Occurrence Remarks (O.R.) of Isolated Fungi from 54 Asthmatic and 34 Patients with Acute Tracheobronchi-tis.

Species isola	Specimens					
	Mouth Swabs (87)			Sputum Spec. (53)		
	No.	%	O.R	No.	%	O.R
Asperg. fumigatus	5	15.1	L	8	35	L
Aspergillus niger	3	9.1	R	5	22	L
Aspergillus flavus	2	6.1	R	1	4	R
Candida Sp.	15	45.5	H	4	18	R
Cladosporium sp.	5	15.1	L	2	9	R
Penicillium rubrum	3	9.1	R	1	4	R
Penc. chrysogenum	—	—	—	1	4	R
Fusarium oxysporum	—	—	—	1	4	R
Total positives	33	100		23	100	
Negative samples	54			30		
% of negatives	62			56		
% of positives	38			44		

H = High occurrence (more than 10 positives).

L = Low occurrence (positives between 5-10 cases).

R = Rare occurrence (positives less than 5 cases).

Table (4) : Effect of Medicinal Plant Extracts on Growth of Some Fungi After 8 Days at 28°C.

Species	Plant extracts				
	Allium sativum	A.cepa	Cinn. cassia	Zingber officinale	Cuminum cyminum
Aspergillus fumigatus	+++	++	+	++	+
A.niger	++	++	+	++	+
A.flavus	++	+	+	+	—ve
Candida sp.	++++	+++	++	++	+
Cladosporium sp.	++	++	+	++	+
Penicillium rubrum	+++	+	++	++	++
P.chrysogenum	++	+	+	+	+
Fusarium oxyporum	++	++	+	++	+

Discussion

Fungal diseases are referred to as mycoses. Some mycoses are endemic[13]. Most endemic mycoses are acquired by the inhalation of Candida. Some mycoses are chiefly opportunistic granulocytes dysfunction (quantitative or qualitative), so depressed cell-mediated immunity (CMI), and diabetic keto-acidosis, predispose to mycosis[13].

The pulmonary syndromes associated with fungi in man are asthma, hypersensitivity pneumonitis, saprophytic colonization and infection[14]. Growth of fungi indoors may cause problems for asthmatics, and occasional outbreaks of fungal disease, may result from careless removal of air-filters in operating theatres, or from fungal growth in air conditioners[15,16]. Awareness and improvement of diagnostic mycological technics showed the increased prevalence of pulmonary mycosis[12]. Pulmonary mycosis should be considered when patients receiving corticosteroids develop a pneumonia that fails to respond to broad spectrum antibiotics[17].

The present study included 87 patients. Fifty three out of 87 were asthmatics, the remaining were patients with acute tracheobronchitis. A mouth swab and a sputum specimen were taken from each asthmatic patient for isolation and identification of fungi then we studied the sensitivity of these isolated fungi to some medicinal plant extracts. Cultures of 27 mouth swabs and 23 sputum specimens, out of 53 collected from asthmatics were positives, (51% & 43% respectively) :

In asthmatic patients under bronchodilators with corticosteroids, 80% & 90%

of their mouth swabs and sputum specimens respectively were positive to different types of fungi. While in asthmatics under bronchodilators without steroid therapy positivity of fungal isolation were 44 & 33% for mouth and sputum specimens respectively. Other investigators reported higher incidence of fungi in their patients whom under corticosteroid therapy (87% & 71%)[**1,5**] while other workers reported that only 24% of asthmatics had positive sputum culture for fungi[**18**]. These different results in different studies may be related to environmental factors, doses and/or duration of steroid therapy.

In the present work Candida species were isolated in 41% and 18% of mouth swabs & sputum specimens of asthmatics respectively. While Aspergillus species were isolated from 10% & 14% mouth swabs and sputum specimens respectively out of 27 & 23 fungi isolated from 53 asthmatics. Least common fungi that isolated were Cladosporium species, Penicillium rubrum, Penicillium chrysogenum and Fusarium oxysporum. Studied cases of Roderigws et al.[**17**] had 75% Canadida albicans and 67% Aspergillus species. Other investigators[**11,19**] found high positive percentage for Aspergillus species in the sputum of their patients. Geographical distribution of fungi in nature could explain this difference.

As regards patients with acute tracheobronchitis, only their mouth swabs were cultured for fungi as no sputum could be taken. Only 6 patients out of 34 had positive cultures for fungi, and Candida species were the most common (67%), which might be due to bad oral hygiene, and/or abusing antibiotics.

Isolated fungi in the present work were sensitive in different degrees to different medicinal plant extracts used in the study, (Allium sativum, Allium cepa, Cinnamomum cassia, Zingber officinale, and Cuminum cyminum). The most sensitive to most studied plant extracts was candida species followed by Aspergillus species then Penicillium species, and lastly Fusarium oxysporum (table 4).

Conclusion :

Cases with chronic obstructive lung diseases and asthmatics are more prone for pulmonary mycosis, and this should direct our attension to ask for fungal study in the sputa of these patients specially those under steroid therapy. Diligent care of these patients should be considered from fear of invasive pulmonary aspergillosis specially in those with neutropenia [**20,21**].

Medicinal Plant extracts may play a role in the future to eradicate fungi, further studies will be needed.

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