Original article

Airborne fungi in Isfahan and evaluation of allergenic responses of their extracts in animal model

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Abstract

Introduction and objective: Detection of the common allergen airborne fungi in any region is critical for prevention and treatment of allergic fungal diseases. Therefore, this study was conducted to identify the most common airborne fungal species of Isfahan, investigate their allergic reactions in animal and obtain local fungal strains for use as antigens in allergy tests to be used.

Materials and methods: An open plate method was used to scan airborne fungal contents over 12 months in Isfahan. On the same days every week, triplicate samples were collected at three different locations in the morning, at noon and in the evening. The fungal culture media were incubated at 25ºC until growth appeared and then the airborne fungi were identified by routine mycological laboratory methods. The extracts of the most common airborne fungi isolated, were examined with skin prick test for allergic reactions in laboratory animals.

Results: During this study, the most abundant airborne fungi identified in Isfahan were species of yeasts, yeast like (*Candida* spp*., Geotrichum* spp., and *Trichosporon* spp.), and mold (*Cladosporium* spp., *Penicillium* spp*., Aspergillus* spp., and *Alternaria*). Positive skin reactions were observed with *Cladosporium* spp.(40%), and *Aspergillus* spp. (21%), *Alternaria* spp. (17%), *Penicillum* spp. (15%) and *Geotrichum* spp. (7%).

Conclusion: It is concluded that fungi have a significant role in infecting immunocompromised hosts, information obtained in the present study contribute toward a better understanding of the pattern of occurrence of airborne fungi, and may assist allergists, clinicians and epidemiologists to treat the diseases.

Keywords: Airborne fungi, Allergenicity, Skin prick test, Isfahan

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Introduction

Previous studies have shown that airborne fungal spores are very important sensitizing agents in allergic respiratory diseases such as asthma and rhino-conjunctivitis [1-3]. Sensitization to airborne fungal elements has been shown to associate with both asthma severity and death [4,5]. In the recent years, air quality has become an important environmental health issue which in part is related to allergen airborne fungal contaminations.

All atmospheric air, whether indoor or outdoor, contains certain varieties and some fungal spores. The concentration of the fungal elements differs according to location, altitude, time of day, season and climatic conditions [3,6,7]. Therefore, fungal allergy sufferers are always exposed to fungal elements, and what differentiates exposure in one area from another is the species of allergen airborne fungi and their quantity of spores in the air [1,3]. Also, many different airborne fungi can act as etiological agents of otomycosis, keratomycosis, onychomycosis, acute respiratory mycoses and chronic bronchitis [3,8,9].

Due to increasing awareness of the relationship of airborne fungi to allergy, scientists and allergists began to study the spectrum and incidence of airborne fungi worldwide [6,7,10-13]. Detection of the common allergens in any region is critical in the prevention and treatment of allergic diseases. Allergy skin test materials are not available for most airborne fungi in Iran; those available are imported and not standardized with domestic fungal strains. Yet, the practicing allergist and clinical immunologist must select what fungal extract are available based on the knowledge of air sampling data and personal experience of the patient in each specific area.

The goals of this study were to determine the prevalence of airborne fungi in Isfahan and to investigate their allergic reactions in laboratory animal to further obtain local fungal strains for use as antigens in allergy tests.

Materials and methods

An open plate method was used to scan airborne fungal contents over 12 months. The triplicate sampling was carried out in three different locations on the same days every week in the morning, at noon and in the evening [14]. The fungal culture media used, Sabouraud dextrose agar containing chloramphenicol (SC, Merck, Germany), and Potato dextrose agar, (PDA, Merck, Germany) were incubated at 25ºC until growth appeared. The airborne fungi were identified by standard mycological procedures {slide culture for filamentous fungi and API 20C system (BioMérieux SA, France) for yeasts} [8,9].

Stock culture of local isolated airborne fungi were maintained in chloramphenicol SC and inoculate were taken from them and grown in SC broth. At the end of incubation period, the mycelia mat was separated from the medium, dried and grounded to a powder and crud extraction was prepared according to modified method of Budd *et al.* [15]. Also the SC broth, in which the fungus was grown, concentrated, dialyzed and then freeze-dried. The crud extract of this material was prepared according to modified procedures similar to that for the preparation of other fungal extracts [15].

The crud extract obtained from both mycelia mat and broth culture were mixed together and centrifuged, sterilized by filtration and then diluted 1:1 with glycerin and stored at 4ºC or lyophilized to preserve allergen potency.

The skin prick test was performed by making a controlled superficial cut into the epidermis of the back of rabbits and a drop of antigen was applied to the test site [16]. The degree of erythema and wheal formation measured after 15-20 minutes and compared with positive (histamine) and negative (glycerol buffer) controls. The erythema reaction ≥10 mm was considered as positive allergenic reaction.

Results

In this study, 250 samples from air in Isfahan were taken and 828 colonies were isolated. The genera of isolated airborne fungi, depending upon their frequency in number of colony counts were classified as predominant and less frequent isolates. The dominant species were members of the genera *Penicillium* spp., *Cladosporium* spp., *Aspergillus* spp., *Alternaria* spp. and yeasts and yeast like (*Candida* spp., *Geotrichum* spp. and *Trichosporon* spp.) and the minor components or less prevalent were *Rhizopus* spp., *Ulocladium* spp., *Curvularia* spp. and *Fusarium* spp. (Table 1). Seasonal variations of the total colony counts were significantly ($P<0.05$) different. They showed higher concentrations in the autumn and winter seasons and the lowest in summer.

The extracted materials prepared from airborne fungal species are assumed to contain no dialyzable mould metabolites, including some of the allergens. These extracts were tested in 20 rabbits by prick test; all rabbits showed positive reactions to various fungal extracts with different percentages (Table 2). The positive skin reactions were observed with *Cladosporium* spp. (40%), *Aspergillus* spp. (20%), *Alternaria* spp. (20%), *Penicillium* spp. (15%) and *Geotrichum* spp. (5 %).

Table 1: The frequency of airborne fungi isolated from Isfahan

Fungal genera	Total no. of colonies	%
Penicillium spp.	327	39.5
Cladosporium spp.	146	17.7
Yeast spp.	155	18.7
Aspergillus spp.	107	13.0
Alternaria spp.	57	6.9
Rhizopus spp.	10	1.2
Ulocladium spp.	6	0.7
Curvularia spp.	5	0.6
Fusarium spp.	4	0.5
Scopulariopsis spp.	3	0.3
Acremonium spp.	2	0.2
Helminthosporium spp.	1	0.1
Unknown moulds	5	0.6
Total	828	100

Table 2: Percentage of positive skin reactions to common isolated airborne fungi

Fungal extract	No. of rabbits tested positive	Skin reaction (%)
Cladosporium spp.	8	40
Aspergillus spp.	4	20
Alternaria spp.	4	20
Penicillium spp.	3	15
Geotrichum spp.	1	5

Discussion

The role of fungi in allergic diseases is well documented. Airborne pollen and fungal allergenic spores have been implicated as one of the main cause of allergic respiratory diseases in temperate regions [1,3,10,11]. The dominant species of airborne fungi throughout the year in Isfahan's atmosphere were *Cladosporium* spp., yeasts and yeast like (*Candida* spp., *Geotrichum* spp. and *Trichosporon* spp.), *Penicillium* spp., *Aspergillus* spp. and *Alternaria* spp. These results are comparable to those from a previous study [14]. Reports and surveys of allergen airborne fungi from almost all parts of the world now appear in the literature. Studies in North, South and Central America have shown that *Cladosporium* spp, *Penicillium* spp., *Aspergillus* spp. and *Alternaria* spp. are the prevalent airborne fungi [11,13].

Other studies in Germany [12] have shown the *Penicillium* and *Cladosporium* spp. and in Sudan [7] *Cladosporium* spp. are predominant. The report from Greece stated that *Cladosporium* spp., *Alternaria* spp., and *Ustilago* spp. were the most common allergens in patients with respiratory allergy [5]. Allergens dispersed by airborne fungi play an important role in the underlying cause and exacerbation of allergic diseases [1,3]. The most common fungal allergens in our neighbouring countries near Iran, such as Iraq, Kuwait, Turkey and Saudi Arabia have been reported as *Alternaria* spp., *Aspergillus* spp., *Cladosporium* spp., *Penicillium* spp. and *Ulocladium* [6,17-19]. Although these observations are somewhat similar to our results they are different in seasonal patterns, fluctuation and prevalence.

The differences are probably due to interacting environmental factors, such as climate, vegetation and geographic location. Hasnain *et al.* [20] found a close relationship between the seasonal fluctuation of airborne basidiospores and the pattern of acute asthma in the clinics in New Zealand. The researchers in Canada have examined the fungal spores of *Alternaria* spp., *Aspergillus* spp., *Cladosporium* spp. and *Penicillium* spp. in the homes of patients who had allergic rhinitis or asthma and found the high counts of these fungi [21]. In addition to the airborne fungi, the thermophilic *Actinomycetes* spp. is also capable to cause allergy in sensitive subjects [22].

It is believed that an individual can develop an allergy to certain fungal strains for which he or she had not previously displayed an allergic reaction, even if some of these fungi may not have been known previously to cause allergy in human [1,3]. The present study revealed that hypersensitivity to *Cladosporium* spp., *Aspergillus* spp., *Alternaria* spp., *Penicillium* spp. and *Geotrichum* spp. was common and these airborne allergen fungi are identified to be the risk factors for allergic diseases in Isfahan. These findings

necessitate further investigation as regards the purification and characterization of these local extracts for diagnostic skin test and prophylaxis of allergic diseases due to airborne fungi in Isfahan.

Although the pattern of allergen fungi in Isfahan was somehow similar to the species of our neighbouring countries [6,17-19] but some strains did not produce positive allergic reactions in animal. This may be explained by the findings of researchers that many fungal allergen contents exhibit substantial micro heterogeneity with respect to both molecular weight and isoelectric point [10,23, 24]. In addition, most common genera have a large number of species, and there are distinct strains within them. Therefore, the practicing allergist and clinical immunologist must select what fungal extract are available based on the knowledge of air sampling and personal exposure of the patient in each specific area which was the goal of our study.

Researchers in Saudi Arabia found that antigens extracted from local strains of *Cladosporium* spp, *Penicillium* spp., *Aspergillus* spp., *Alternaria* spp., *Ulocladium* spp., *Drechslera* spp., and *Stachybotrys* spp. reacted positively in 13% of patients having asthma, revealing allergic sensitization to these fungi [23]. In Singapore, Chew *et al.* [10] found 26-32% positive reactions to local extracts of *Drechslera* spp. and *Curvularia* spp. and 32% to common fungal spores in allergic patients in Greece [5].

Conclusion

Beside the role of these fungi in infecting immunocompromised hosts, information obtained in the present study contribute toward a better understanding of the pattern of occurrence of allergen airborne fungi, and may be valuable for clinicians, allergists and epidemiologists.

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