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Fungal Pollution in the Homes of Respiratory Allergic Patients in Mashhad City, Northeast Iran

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Abstract

Background: Fungal spores are ubiquitous constituents indoors and outdoors and are now generally identified as important causes of respiratory allergies.
Objectives: This study aims to evaluate the frequency and prevalence of allergens with fungal origin at homes of allergic patients with respiratory symptoms in the city of Mashhad, Iran.
Methods: In this cross-sectional study, a group of patients with allergic rhinitis (n = 50) were selected based on positive skin prick test. Healthy volunteers with no respiratory allergy were included in control group with the same age as diseased group. Samples from nasal cavity and different parts of bedroom were collected and cultured. The fungal agents were recognized by conventional mycological methods based on the cultural and microscopic appearance as properties.
Results: Colonization with fungi was obtained in 26% - 64% and 86-98% of the patient and healthy groups, respectively. Among all species isolated from specimens of the patients, *A.flavus* was dominant which followed by *A. niger*, *Penicillium* and *Cladosporium* whereas the most commonly isolated fungi from the whole specimens of healthy subjects were *A. flavus*, *A. fumigatus*, *A. niger*, *Penicillium*, *Yeast*, *Alternaria* and *Cladosporium*.
Conclusions: Fungi can be hazardous for health. Furthermore, prevention of fungal spores' growth is important, and also identifying and determining the most common allergen fungi are key steps to provide the necessary recommendations to the patients in controlling and preventing disease.

Keywords: Fungal Allergens, Allergic Rhinitis, Healthy Volunteers, Indoors Pollution

1. Background

The incidence of fungal diseases has risen rapidly over the last two decades, and fungal allergy is one of the common health problems/ medical conditions worldwide (1). The prevalence of respiratory allergies caused by fungi is estimated at 20% to 30% among the individuals with atopic history and up to 6% in general population (2). The viability and replication of fungi in nasal and sinus mucus are the same as well as indoor environments that these characteristics differentiate them from other allergen classes. Fungal spores widely exist in nature, and it is estimated that 3 to 10% of the world's population are allergic to molds, but demonstrate unique regional trends by reason of latitude, climatic differences, humidity, and probably other factors such as vegetation. The substrate for fungal growth is another important variable in indoor environments (3).

Since about the middle of the 20th century, the prevalence of allergic diseases such as allergic rhinitis (AR) has been increasing all over the world (4). AR is characterized by paroxysms of sneezing, rhinorrhea, and nasal obstruction, often accompanied by itching of the eyes, nose, and palate (5). AR is an upper respiratory disease caused by immunoglobulin E (IgE) interference following contact with allergens (6). The catalogue of the fungal allergens has recently described lists including 174 allergens for the genus Ascomycota and 30 for the genus Basidiomycota. Several epidemiological, environmental, and clinical studies were focused on the relevant species like *Alternaria*, *Aspergillus*, *Cladosporium* and *Penicillium* (7). Airborne allergens with fungal origin have been implicated as one of causes of allergic respiratory diseases in our region and neighbor countries (8).

2. Objectives

Because of diverse distribution and pattern of airborne allergens in different regions, identification and determination of common allergens is essential for the appropriate diagnosis of prophylaxis and treatment of the patients suffering from the disease. The current study was conducted to evaluate the frequency and prevalence of fungal allergens at homes of the patients with allergic rhinitis in Mashhad to develop better strategies for prevention, management and treatment of allergic rhinitis.

3. Methods

In this cross-sectional study, the study population was a group of 50 patients with allergic rhinitis who visited the allergic clinics in Mashhad, Iran during 2016, and their prick skin testes were positive. A group of 50 volunteers (with no respiratory allergy) who were in similar age groups included as control group. The study population completed the informed consent form to participate in the research, and it was approved by the ethics committee of Mashhad University of Medical Sciences. The research participants had resided in sampled houses for at least 1 year before the study. Samples were collected from nasal cavity and bedroom of healthy volunteers and patients with allergic rhinitis.

Sampling sites in the sleep environment include indoor air, pillow, dust of the bed and dust on the shelf. Sampling from the dust samples was done when the bedroom had not been cleaned for at least 48 hours. The airborne fungal spores were collected by sedimentation method. This method is commonly employed by various investigators (9, 10). Opened plates (three plates per bedroom) containing Sabouraud dextrose agar (Merck Co., Darmstadt, Germany) supplemented with chloramphenicol (SC) were held exposed for each bedroom for 15 min. To minimize indoor contamination by outdoor molds, we collected samples while the windows were closed. Nasal cavity and dust samples were collected using sterile cotton swabs moistened with sterile saline solution (0.9 % NaCl); then swabs were seeded onto the surface of SC. All plates were incubated at 27°C for 7 - 14 days. The fungal agents were recognized by conventional mycological methods based on cultural and microscopic appearance as properties.

3.1. Statistical Analyses

Fisher's exact and Chi-square tests were used to compare the frequency of fungi in nasal cavity and different parts of bedroom. All the samples were taken from healthy volunteers and patients with allergic rhinitis. All calculations were performed using SPSS software version 21 and P value less than 0.05 was statistically considered significant.

4. Results

15 (30%) females and 35 (70%) males were in the group of healthy volunteers. The age range of them was 15 to 67 years (median 30). Out of all the patients enrolled, 30 (60%) were females and 20 (40%) were males. Their age range varied from 18 to 61 years (median 27). A total of 500 plates were collected from both groups, of which 100 were nasal cavity samples and 400 were bedroom samples. In the control group, colonization with fungi was obtained from the samples in all 50 bedrooms including 46 (92%) out of the 50 nasal cavity samples, 49 (98%) bedroom air samples, 47 (94%) pillow samples, 43 (86%) dust of the bed samples, 47 (94%) dust on the shelf samples. In the patient group, colonization with fungi was obtained from the samples in all 50 bedrooms including 32 (64%) out of the 50 nasal cavity samples, 30 (60%) bedroom air samples, 18 (36%) pillow samples, 24 (48%) dust of the bed samples, 13 (26%) dust on the shelf samples.

In Table 1, we present the frequency of different fungi isolated from nasal cavity and different parts of bedroom in control and patient groups. The most commonly isolated fungi from the whole specimens of the healthy subjects were *A. flavus*, *A. fumigatus*, *A. niger*, *Penicillium*, *Yeast*, *Alternaria* and *Cladosporium*; whereas *Fusarium*, *Stemphylium*, *Ulocladium*, *Stachybotrys* and *Exophiala* showed the least frequencies among the isolated fungi. Among all the species isolated from specimens of the patients, *A. flavus* was dominant which followed by *A. niger*, *Penicillium* and *Cladosporium*; whereas *Scopulariopsis* and *Stemphylium* showed the lowest frequencies.

A significant correlation was reported between the frequency of *A. fumigatus* and *A. niger* isolated from nasal cavity samples and healthy volunteers, compared with the patients ($P < 0.05$).

In the bedroom air samples, the frequency of all species of *Aspergillus*, *Penicillium*, *Paecilomyces*, *Mucor*, *Rhizopus*, *Cladosporium* and *Yeast* had significant correlation with healthy volunteers, compared with patients ($P < 0.05$). Furthermore, there was a statistical difference in frequency of *A. flavus* and *Penicillium* isolated from pillow samples between healthy volunteers and patients ($P < 0.001$). Regarding dust of the bed samples, a significant difference was observed between the frequencies of *A. fumigatus*, *Penicillium*, *Alternaria* and *Yeast* of control group compared with the patients ($P < 0.05$). Also, the frequency of *A. niger* and *A. fumigatus* isolated from dust of the shelf samples had a significant correlation with both healthy volunteers and patients ($P < 0.05$).

Table 1. The Frequency of Fungi Isolated from Nasal Cavity and Different Parts of Bedroom in Control and Patient Groups[a]

Fungal Genera	Nasal Cavity		Bedroom Air		Pillow		Dust of the Bed		Dust of the Shelf		Total Bedroom Environment	
	Control	Patient	Control	Patient	Control	Patient	Control	Patient	Control	Patient	Control	Patient
A. flavus	12 (24)	13 (26)	29 (58)	11 (22)	21 (42)	4 (8)	16 (32)	8 (16)	5 (10)	2 (6)	40 (80)	22 (44)
A. niger	10 (20)	3 (6)	23 (46)	5 (10)	6 (12)	2 (6)	6 (12)	4 (8)	15 (30)	4 (8)	34 (68)	15 (30)
A. fumigatus	16 (32)	1 (2)	12 (24)	1 (2)	1 (2)	2 (4)	14 (28)	0 (0)	21 (42)	0 (0)	33 (66)	3 (6)
Penicillium	6 (12)	8 (16)	13 (26)	4 (8)	28 (56)	1 (2)	7 (14)	1 (2)	6 (12)	1 (2)	38 (76)	7 (14)
Paecilomyces	2 (4)	2 (4)	5 (10)	0 (0)	3 (6)	1 (2)	2 (4)	1 (2)	0 (0)	0 (0)	10 (20)	2 (4)
Acremonium	0 (0)	0 (0)	0 (0)	0 (0)	1 (2)	0 (0)	1 (2)	0 (0)	0 (0)	0 (0)	2 (4)	0 (0)
Fusarium	0 (0)	0 (0)	0 (0)	1 (2)	1 (2)	1 (2)	0 (0)	2 (4)	0 (0)	0 (0)	1 (2)	4 (8)
Scopulariopsis	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (2)	1 (2)	0 (0)	1 (2)	0 (0)	2 (4)	1 (2)
Geotrichum	1 (2)	0 (0)	0 (0)	0 (0)	1 (2)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (2)	0 (0)
Mucor	0 (0)	0 (0)	10 (20)	0 (0)	1 (2)	0 (0)	2 (4)	0 (0)	1 (2)	0 (0)	13 (26)	0 (0)
Rhizopus	2 (4)	0 (0)	4 (8)	0 (0)	3 (6)	0 (0)	1 (2)	0 (0)	1 (2)	0 (0)	6 (12)	0 (0)
Alternaria	3 (6)	0 (0)	4 (8)	1 (2)	2 (4)	0 (0)	12 (24)	2 (4)	4 (8)	1 (2)	19 (38)	4 (8)
Cladosporium	1 (2)	3 (6)	13 (26)	3 (6)	0 (0)	2 (4)	3 (6)	3 (6)	3 (6)	2 (4)	16 (32)	10 (20)
Stemphylium	0 (0)	0 (0)	0 (0)	1 (2)	0 (0)	0 (0)	0 (0)	0 (0)	1 (2)	0 (0)	1 (2)	1 (2)
Ulocladium	0 (0)	0 (0)	1 (2)	2 (4)	0 (0)	1 (2)	0 (0)	0 (0)	0 (0)	1 (2)	1 (2)	4 (8)
Stachybotrys	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (2)	0 (0)	1 (2)	0 (0)
Exophiala	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (2)	0 (0)	0 (0)	0 (0)	1 (2)	0 (0)
Yeast	5 (10)	2 (4)	18 (36)	2 (4)	2 (4)	2 (4)	14 (28)	2 (4)	7 (14)	1 (2)	29 (58)	7 (14)
Total	58	32	132	31	70	17	80	23	66	12	248	80

[a] Data are presented as No.(%).

5. Discussion

Fungal spores are ubiquitous constituents indoors and outdoors and are now generally identified as important causes of respiratory allergies. More than 80 genera of fungi have been associated with both upper (rhinitis) and lower (asthma) symptoms of respiratory tract allergy (11).

In our study, morphologically, 78.65% of the isolates from both groups belonged to 4 genera: *Aspergillus*, *Penicillium*, *Cladosporium* and *Alternaria fungi* which are commonly considered allergenic; these findings are in agreement with the findings of several other researchers (12-19). In the current study, fungi were isolated from 92% and 64% nasal cavity of healthy volunteers and patients with allergic rhinitis, respectively. In a similar research to ours, Sellart-Altisent et al. reported that fungi were isolated from 41.5% of healthy people and 14.8% of allergic patients (20). Moreover, Arabi-Mianroodi et al. showed that the fungi were detected from nasal mucosa of 31% healthy adults (21). Darwazeh et al. isolated fungi from the nose of 21% Saudi healthy subjects (22). In contrast, Khazraei et al. revealed that the fungi from culture medium of nose excretion were isolated from 15 (24%) cases and 5 persons (8%) in control group (23). Furthermore, Fathy et al. showed positive fungal culture of nasal secretions in 12 patients with allergic rhinitis (30%) and 2 cases (10%) in healthy subjects (24). The lower prevalence of fungi in nasal mucosa of allergic patients in our study could be related to the nasal inadequacy, the hypersecretion and the larger use of handkerchiefs (20).

According to Table 1, *Aspergillus* (68%), *Penicillium* (12%), *yeast* (10%) and *Alternaria* (6%) were the most frequently isolated fungi from nasal cavity of healthy volunteers; whereas, the most dominant fungi isolated from nasal cavity of patients were *Aspergillus* (34%), *Penicillium* (16%) and *Cladosporium* (6%), respectively. In this regard, Arabi-Mianroodi et al. in Kerman, Iran showed that the fungi isolated from nasal mucosa of healthy adults were with a frequency rate as follows: *yeast* (12%), *Aspergillus* (8%), *Penicillium* (3%) and *Mucor* (1%) (21). Khazraei et al. in Shahrekord, Iran reported that the most common isolated fungi from nose excretion of the patients with allergic rhinitis were *Aspergillus* (8%) and *Penicillinum* (6.5%), whereas, in the patients without allergic rhinitis, *yeast*, *Penicillinum* and *Rhizopus* were respectively isolated (23). Kordbacheh et al. in Tehran, Iran isolated *A. flavus* (7%), *A. fumigatus* and *Rhizopussp.* (each 1%) from the patients with nasal polyposis (25). Likewise, Darwazeh et al. reported that *A. flavus* was dominant species isolated from Saudi healthy subjects that followed by *A. niger* (31.8%) and *A. fumigatus* (26.9%) (22). Sellart-Altisent et al. in Spain showed that the most commonly isolated genus from nasal cavity of allergic and healthy subjects was *Cladosporium*, followed by *Penicillium*, *Aspergillus* and *Alternaria* (20).

In the current study, fungi were isolated from different four parts of bedrooms including indoor air (60% vs. 98%), pillow (36% vs. 94%), dust of the bed (48% vs. 86%), and dust on the shelf (26% vs. 94%) in control and patient groups, respectively. The lower prevalence of fungi in different parts of bedrooms of allergic patients in our study could be related to their hypersensitivity to keep clean homes to reduce allergens; however, they may be sensitive to continuous low-dose exposure of fungal allergens in indoors.

In the present study, the most abundant fungi isolated from all parts of bedroom of 50 healthy volunteers were *Aspergillus* (98%), *Penicillium* (80%), *yeast* (58%), *Alternaria* (36%) and *Cladosporium* (32%), respectively; whereas, *Aspergillus* (68%), *Cladosporium* (20%), *Penicillium* and *yeast* (14% each) were the most common fungi isolated from all parts of bedrooms of 50 patients. Several studies have investigated the isolation and frequency of allergen fungi in homes of healthy subjects and asthmatic/rhinitis patients (12-17). The studies performed in different countries provide variable results of total fungal concentration and distribution of fungal species because it basically depends on media and sampling method used, season of the year, geographical location, and living conditions as well as fungal growth substrates in different countries (26). In any case, the presence of fungi, particularly allergen fungi, in indoor environments of healthy/allergic patients' homes may have adverse effects on human health and/or may cause allergic symptoms are getting worse. Internal factors such as warm and moistened conditions, poorly maintained heating, ventilation and air-conditioning (HVAC) systems, changing of key factors including life-style habits and the construction of buildings with new materials as well as external factors such as increased pollution have been recognized as important causes of growth and distribution of indoor fungi (17).

In conclusion, this study showed that fungi can be hazardous for health. Furthermore, prevention of fungal spores' growth is important, i.e. the reduction of heat and moisture in indoor environments, and also identifying and determining the most common allergen fungi are key steps to provide the necessary recommendations to patients in controlling and preventing disease. Likewise, more researches are needed to understand the reasons for less isolation of fungi in allergic patients by studying the large numbers of patients, evaluating the different variables, etc.

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