Original Article

Zahedan Journal of Research in Medical Sciences

Journal homepage: www.zjrms.ir

Isolation of Aspergillus species from Nasal Cavity and Bedroom of Healthy Volunteers and Patients with Allergic Rhinitis in Mashhad, Iran

Samaneh Eidi,*[1] Abdolmajid Fata,[2] Reza Farid-Hosseini,[3] S. Amir Kamali,[1] Zahra Hajari,[1] Ali Naseri,[2] Mehdi Bakhshaee[4]

1. Department of Pathobiology, Faculty of Veterinary Medicine, Ferdowsi University of Mashhad, Mashhad, Iran
2. Department of Medical Mycology and Parasitology, Mashhad University of Medical Sciences, Mashhad, Iran
3. Bu-Ali Immunology Research Center, Mashhad University of Medical Sciences, Mashhad, Iran
4. Department of Otorhinolaryngology, Head and Neck Surgery, Mashad University of Medical Sciences, Mashhad, Iran

Article information

Article history:
Received: 7 Oct 2013
Accepted: 21 Dec 2013
Available online: 22 Jan 2014
ZJRMS 2014 Nov; 16(11): 15-19

Keywords:
Aspergillus spp.
Allergic rhinitis
Nasal cavity

*Corresponding author at:
Department of Pathobiology,
Faculty of Veterinary Medicine,
Ferdowsi University of Mashhad,
Mashhad, Iran.
E-mail:
eidi@ferdowsi.um.ac.ir

Abstract

***Background*:** The purpose of this study was to investigate the presence, frequency and comparison of Aspergillus spp. in nasal cavity and bedroom of healthy volunteers and patients with allergic rhinitis.

***Materials and 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 were chosen to be in the comparison group by matching in age, gender, and no history of respiratory system disease. Samples from nasal cavity and different parts of bedroom were collected and cultured. Cultured Aspergillus spp. was identified by standard mycological techniques.

***Results*:** The most common species isolated from all samples of healthy volunteers was *A. flavus* (88%), followed by *A. niger* (76%) and *A. fumigatus* (74%). *A. flavus* (56%) was the predominant species isolated from all samples of patients, followed by *A. niger* (34%) and *A. fumigatus* (6%).

***Conclusion*:** *A. flavus* was the most prevalent species of Aspergillus both healthy volunteers and patients. The presence of Aspergillus in homes does not necessarily imply a cause and effect relationship with illness, but we speculate that *A. flavus* may be a major source of aeroallergens along with *A. niger* and *A. fumigatus*; and should alert physicians and healthcare professionals to do more vigorous environmental testing.

Introduction

Fungi are common in both outdoor and indoor environments as spores, mycelial fragmentation or dissociated intracellular and extracellular components. They are usually saprophytic and harmless but under extra ordinary conditions such as lung diseases or weakened immune systems, they can cause infections indifferent spectrum of illness includes allergic reactions, lung infections, and infections in other organs [1].

It has been reported that the increasing incidence of allergic diseases, such as asthma, rhinitis and atopy, is caused by molds in the indoor environment [2]. Therefore, indoor air quality has become an important health concern. Considering the fact that an allergic reaction may occur with exposure to minute concentration of an allergen [3], indoor molds could create a health risk for atopic individuals occupying such a building.

More than 80 genera of fungi have been associated with symptoms of respiratory tract allergies [4]. Aspergillus can be a part of indoor mycoflora. Several species of Aspergillus have been shown to be allergenic, including *A. fumigatus*, *A. niger*, *A. flavus*, and *A. oryzae* [5]. In susceptible individuals or those exposed to extremely high inocula, invasive aspergillosis may follow Aspergillus exposure. It has been demonstrated previously that individuals can and do become sensitized to Aspergillus allergens [6]. The aim of this study was to investigate the presence, frequency and comparison of Aspergillus spp. in nasal cavity and bedroom of healthy volunteers and patients with allergic rhinitis.

Materials and Methods

In this cross-sectional study, the study population was a group of 50 patients with allergic rhinitis whose prick testes were positive and referred to allergic clinics during April-August 2012. A group of 50 volunteers who appeared to be healthy and had no history of respiratory system disease and were in similar age and gender groups included as control group. An informed consent form obtained from all individual of each group. Samples were collected from nasal cavity and bedroom of healthy volunteers and patients with allergic rhinitis as follow:

1- Healthy volunteers: After giving informed consent. Subjects had resided in sampled houses for at least 1 year before the study.

2- Patients: Fifty patients clinically diagnosed as allergic rhinitis and approved by Prick skin test were enrolled in this study. The patients completed the consent form to participate in the research, and it was approved by the ethics committee of Mashhad University of Medical

Sciences. Patients had resided in sampled houses for at least 1 year before the study.

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 [7, 8]. Opened plates (three plates per bedroom) containing Sabouraud dextrose agar 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 25-27ºC for 7-14 days. The fungi were identified by standard mycological techniques based upon gross cultural and microscopic morphology. The fungi that could not be identified by this manner were subcultured on potato dextrose agar, water agar, and/or slide cultures for further study. Each colony suspected of being Aspergillus was subcultured onto Czapek-Dox agar medium (HiMedia, Mumbai, India) and described according to macroscopic and microscopic characteristics of each colony.

χ^2 test was performed using SPSS-17.5, and differences were considered significant at $p<0.05$.

Results

Out of all healthy volunteers enrolled, 15 (30%) were females and 35 (70%) males. The age range of them was 15 to 67 years (median 30). Out of all 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 the genus Aspergillus was obtained in 32 (64%) out of the 50 nasal cavity samples, 45 (90%) bedroom air samples, 28 (56%) pillow samples, 26 (52%) dust of the bed samples, 38 (76%) dust on the shelf samples all out of 50 bedrooms. The most common species isolated from all samples of healthy volunteers was *A. flavus* (88%), followed by *A. niger* (76%) and *A. fumigatus* (74%). In the patients group, colonization with the genus Aspergillus was obtained in 17 (34%) out of the 50 nasal cavity samples, 17 (34%) bedroom air samples, 9 (18%) pillow samples, 12 (24%) dust of the bed samples, 7 (14%) dust on the shelf samples all out of 50 bedrooms. The most common species isolated from all samples of patients was *A. flavus* (56%), followed by *A. niger* (34%) and *A. fumigatus* (6%).

Figures 1 and 2 show the frequency of Aspergillus spp. isolated from nasal cavity and different parts of bedroom in control and patient groups, respectively. In table 1, we present the distribution of Aspergillus spp. isolated from nasal cavity and different parts of bedroom in control and patient groups. The most commonly isolated species in nasal cavity of control group was *A. fumigatus* (32%), followed by *A. flavus* (24%) and *A. niger* (10%). *A. flavus* had the highest rate in the bedroom air and pillow samples (58% and 42%, respectively), followed by *A. niger* (46% and 12%, respectively) and *A. fumigatus* (24% and 2%, respectively). *A. fumigatus* (42%) was the predominant species in dust on the shelf samples, followed by *A. niger* (30%), and *A. flavus* (10%); whereas *A. flavus* was the most abundant species in dust of the bed (32%), followed by *A. fumigatus* (28%) and *A. niger* (12%).

The most commonly isolated species in nasal cavity of patients group was *A. flavus* (26%), followed by *A. niger* (6%) and *A. fumigatus* (2%). *A. flavus* had the highest rate in the bedroom air, pillow and dust of the bed samples (22%, 8% and 16%, respectively), followed by *A. niger* (10%, 6% and 8%, respectively), and *A. fumigatus* (2%, 4% and 0%, respectively). In dust on the shelf, *A. niger* (8%) was the predominant species, followed by *A. flavus* (6%). Overall, there were significant differences in frequency of isolation of *A. flavus* and *A. niger* in all samples of healthy volunteers, compared with all samples of patients ($p=0.001$). Frequency of *A. niger* isolates from bedroom air and dust on the shelf samples had significant correlation in healthy volunteers, compared with patients ($p=0.001$). Furthermore, there was significant difference in frequency of *A. flavus* isolates from bedroom air and pillow samples between healthy volunteers and patients ($p=0.001$). Also, frequency of *A. fumigatus* isolates from nasal cavity and bedroom air samples had significant correlation in healthy volunteers, compared with patients ($p=0.001$).

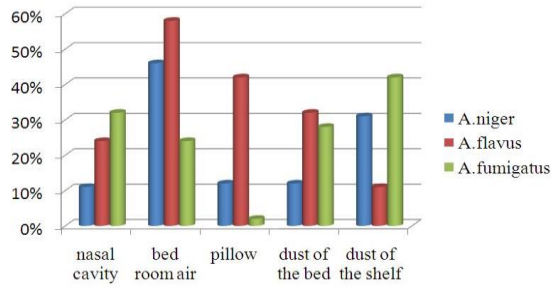

Figure 1. Frequency of Aspergillus spp. isolated from nasal cavity and different parts of bedroom in healthy volunteers

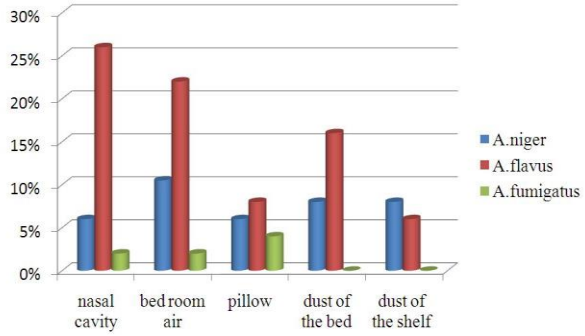

Figure 2. Frequency of Aspergillus spp. isolated from nasal cavity and different parts of bedroom in patients with allergic rhinitis

Table1. Distribution of Aspergillus spp. isolated from nasal cavity and different parts of bedroom in control and patient groups

	Nasal cavity N (%)		Bedroom air N (%)		Pillow N (%)		Dust of the bed N (%)		Dust of the shelf N (%)	
	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)
A. fumigatus	16 (32)	1 (2)	12 (24)	1 (2)	1 (2)	2 (4)	14 (28)	0 (0)	21 (42)	0 (0)
A. niger	5 (10)	2 (6)	23 (46)	5 (10)	6 (12)	2 (6)	6 (12)	4 (8)	15 (30)	4 (8)
Total number of isolates	32	16	64	17	28	8	36	12	41	6

Discussion

In our study, 100% and 70% bedrooms of healthy volunteers and allergic rhinitis patients showed the presence of Aspergillus, respectively. Moreover, the Aspergillus spp. is isolated from 64% and 34% nasal cavity samples of healthy volunteers and allergic rhinitis patients, respectively.

Fungal allergenicity and its role in the pathogenesis of rhinitis are increasingly investigated these days and several allergenic fungi including Aspergillus have been determined [9]. On the other hand, people spend 90% of their time indoors, 50-70% at home, and 30% in the bedroom, almost one third of their life; therefore, indoor air quality has become an important health concern [10]. Many studies have shown that indoor Aspergillus is an important human health risk for those who are susceptible to infection or allergy [5, 6, 11].

Study of Bahkali and Parves revealed that the highest number of fungal colonies were found in the living room followed by bedrooms, and Aspergillus was the predominant genus in house-dusts which corresponds with the results of this study [1]. Su et al. and Sharma et al. noted that Aspergillus had the highest concentration in homes of asthmatic and nonasthmatic children in southern Taiwan and northern India, respectively [12, 13]. Ara et al. showed that the fungal flora in bedrooms was characterized by a high detection rate of xerophilic fungi, such as Aspergillus and Eurotium [2]. Cetinkaya et al. and Hedayati et al. reported that Aspergillus was the second most abundant genus isolated from asthmatic patients' houses and healthy subjects, respectively [4, 10]. Gomez de Ana et al. in Spain exerted that the genus Aspergillus was isolated in approximately 70% (winter) and 89% (summer) of homes of patients allergic to fungi [14].

In contrast, Arabi-Mianroodi et al. isolated Aspergillus only from 8% nasal cavity of healthy individuals [15]. In the present study, the most common species isolated from all samples of healthy volunteers and patients was *A. flavus*, followed by *A. niger* and *A. fumigatus*. *A. fumigatus* was the predominant species isolated from nasal cavity of control group; whereas, in the patient group, *A. flavus* was the most abundant species. In both control and patient groups, *A. flavus* had the highest rate in the bedroom air, pillow and dust of the bed samples; whereas, *A. niger* was the most commonly isolated species from dust on the shelf.

Similar to our findings, Hedayati et al. in north of Iran reported that the most common species of Aspergillus was *A. flavus* both in the indoor and outdoor air of asthmatic patients' houses [10]. In contrast, Sharma et al. in India and Li et al. in Taiwan noted that *A. flavus* and *A. niger* were the predominant species in indoor and outdoor air of asthmatic and control children [13, 16]. Gomez de Ana et al. in Spain showed that *A. niger* was predominantly detected from the indoor air of the homes of patients allergic to fungi [14]. Sakai et al. in Japan reported that the major Aspergillus spp. was *A. restrictus* in indoor and outdoor air of dwellings [17].

Concerning the fungal isolation from nasal cavity, Kordbacheh et al. isolated *A. flavus* and *A. fumigatus* from the patients with nasal polyposis [18]. Likewise, Darwazeh et al. reported that the predominant species isolated from Saudi healthy subjects was *A. flavus* followed by *A. niger* and *A. fumigates* [19]. Sellart-Altisent et al. in Spain revealed that *A. flavus* was the most common species isolated from nasal cavity of allergic and healthy subjects [20].

Unlike the findings obtained from the present study, Woodcock et al. in the UK reported that *A. fumigatus* was the commonest species isolated from pillows and vacuum dust [21].

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 [22].

Several recent epidemiologic studies reported positive associations between home dampness and respiratory morbidity of the occupants. These studies also indicated that dampness and fungal problems are present in 20% to 50% of modern homes. Fungi are regarded as one of the causal factors in the relationship between home dampness and respiratory symptoms, and homes classified as damp tend to have higher levels of fungi than those not so classified. In addition, poorly maintained heating, ventilation, and air-conditioning (HVAC) systems have been recognized as sources of microorganisms, including fungi [23]. Moreover, most fungi, including Aspergillus genus, produce highly allergic proteins or glycoproteins that could cause hypersensitivity diseases in susceptible subjects. There is also evidence suggesting that sensitization and exposure to fungi will increase the chances of asthma attack and some fungi are also clearly associated with symptoms of asthma [12]. Aspergillus spp. has been known to be one of the most prevalent airborne fungi in indoor environment. Exposure to

Aspergillus has been reported to cause several types of human health problems, primarily irritations, infections, allergies, and toxic effects, and it has been suggested that toxigenic Aspergillus are the cause of additional adverse health effects [5, 6, 11]. Several species of Aspergillus have been shown to be allergenic, including *A. fumigatus*, *A. niger*, *A. flavus*, *A. restrictus*, and *A. oryzae*. Over 20 allergens have been characterized in *A. fumigatus*, two from *A. flavus* (Asp fl 13 and Asp fl 18), and four from the closely related *A. oryzae* (Asp o 13, Asp o 21, Asp o lactase, and Asp o lipase) [24, 25]. Microbial volatile organic compounds (MVOC) are low-molecular-weight alcohols, aldehydes, ketones, aromatic compounds, amines, terpenes, chlorinated hydrocarbons, and sulfuric compounds that are produced by either primary or secondary metabolic pathways employing aerobic or anaerobic metabolism [10]. *Aspergillus versicolor* is a strong producer of these compounds. Some other species of Aspergillus including *A. fumigatus*, *A. sydowii*, *A. flavus* and *A. niger* are MVOC producers too [26]. These species are common Aspergillus species that are grown indoors. Some individuals, such as patients with allergic rhinitis, can sense and respond to lower concentrations of MVOC than others.

In addition, *A. flavus*, and *A. fumigatus* are so-called high-risk microorganisms and the confirmed presence of these species will require urgent risk management decisions to be made [12].

In conclusion, *A. flavus* was the most prevalent species of Aspergillus both healthy volunteers and patients. The presence of Aspergillus in homes does not necessarily imply a cause and effect relationship with illness, but we speculate that *A. flavus* may be a major source of aeroallergens along with *A. niger* and *A. fumigatus*; and should alert physicians and healthcare professionals to do more vigorous environmental testing.

Acknowledgements

Authors are grateful to the School of Veterinary Medicine, Ferdowsi University of Mashhad for funding this research (Grant no: 2/16045).

Authors' Contributions

S. Eidi designed the study, supervised all the experimental design, analyzed and interpreted the results and drafted the manuscript. A. Fata contributed in study design, supervised all the experimental design, read and modified the manuscript. R. Farid-Hosseini and M. Bakhshaee supervised the sample collection of patients with allergic rhinitis. SA. Kamali and Z. Hajari contributed in the sample collection of patients with allergic rhinitis and healthy controls and analyzing the results. A. Naseri contributed in the sample collection.

Conflict of Interest

The authors declare no conflict of interest.

Funding/Support

Ferdowsi University of Mashhad.

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***Please cite this article as*:** Eidi S, Fata A, Farid-Hosseini R, Kamali SA, Hajari Z, Naseri A, Bakhshaee M. Isolation of Aspergillus species from nasal cavity and bedroom of healthy volunteers and patients with allergic rhinitis in Mashhad, Iran. Zahedan J Res Med Sci. 2014; 16(11): 15-19.