

Respiratory allergies in Venezuela: are fungi responsible?

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Summary

Exposure to fungi in the indoor environment may trigger hypersensitivity to a variety of fungi and is known to be an influencing factor in allergic rhinitis and asthma. A wide list of airborne fungal spores and dust containing fungi have been described for different environments; however, their clinical relevance is seldom clear. In this survey we measure levels of fungi indoor and outdoor of domestic dwellings of 10 patients with known chronic allergic respiratory disease to fungi. To measure hypersensitivity to fungi, Prick (sensitivity to fungi), RAST (specific serum IgE levels) and PAR (persistent allergic rhinitis) severity are assessed in relation to fungal load in the environment. Only association of PAR and indoor fungal load were found to be significant ($P = 0.1648$). No direct causality with sensitivity to the amount of exposure, or a hypersensitivity to a specific fungal genus could be established. There is still no consensus on the most relevant methods for measuring personal exposure and 'no safe levels' have been established yet.

Key words: respiratory allergies, fungi, Venezuela.

Introduction

The occurrence of dampness and mould in houses has been associated with an increased prevalence of respiratory symptoms.¹ Mould may be implicated in the development of asthma through the production of allergens, toxins and/or volatile components,² and other non-specific inflammatory mechanisms promoted by exposure to mould cell wall products.³ Dampness is associated with respiratory symptoms through sensitisation to dust mite and moulds.⁴ Numerous studies have been undertaken worldwide and more than 80 environmental mould genera have been associated with respiratory system disorders^{5–8} *Cladosporium* spp., *Aspergillus* spp. and *Penicillium* spp. are the most frequently described genera.⁹ Even though there are few reports in Venezuela,^{10–13} the results obtained herein are similar to those found elsewhere in temperate

climate.¹⁴ Because of the presence of allergens on spores it appears that all moulds have the potential to cause an allergic reaction in susceptible humans. The relationship between elevated concentrations of inhaled fungal spores, moulds antigens and induction of respiratory allergy (rhinitis and asthma) is well recognised, although its role in the pathogenicity of these diseases is still unclear.¹⁵ Therefore, the aim of this study was to establish the sensitivity to different moulds in a cohort of respiratory allergic patients from Caracas and to assess the relationship between patient's hypersensitivity to moulds and airborne fungal presence indoor and outdoor at patient's domestic dwellings. To measure hypersensitivity to fungi, Prick (Sensitivity to fungi), RAST (Specific serum IgE levels) and PAR (persistent allergic rhinitis) severity are assessed in relation to fungal load in the environment by sedimentation in agar plates and RCS Plus (aeroscope).

Materials and methods

Study subjects

Over a 1-year period (2002), a total of 139 individuals with respiratory allergy and suggestive clinical history

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Level	Low	Medium	High	Very high	Extremely high
Fungal concentration	<100	101–300	301–1000	1001–5000	>5000 (CFU m ⁻³)

CFU = colony-forming units.

Table 1 Ranges of environmental fungal concentration in CFU m⁻³ according to Platt *et al.*

of sensitivity to fungi were selected from the Allergic Diseases output patient clinical unit of the Instituto de Inmunología, Universidad Central de Venezuela, Caracas. Out of these 10 patients were chosen according to the following inclusion criteria: (a) age range 6–55 years; (b) diagnosis of PAR and/or mild or moderate persistent extrinsic bronchial asthma (BA); (c) higher sensitivity reaction to fungi by prick test; (d) fungal growth at patients housing because of shortcomings in construction; (e) without H1 and/or H2 antihistamines or/and cromoglicate treatment, 48 h before determining serum-specific IgE; and (f) negative stool tests for intestinal parasites.

A control group of 10 healthy voluntary individuals, sex and age matched, were selected from the laboratory staff of the Instituto de Inmunología, contemplating the following inclusion criteria: (a) normal total IgE serum levels (0–100 IU ml⁻¹); (b) normal relative eosinophils count in peripheral blood (0–4%); (c) negative prick test for aeroallergens and proven fungi; (d) absence of allergy antecedents and treatment for other diseases; and (e) negative stool tests for intestinal parasites. The study was submitted and approved by the ethical committee of the institute.

Clinical history

The patient's clinical manifestations and demographic data were compiled in a computerised sheet. The environmental data were collected making emphasis in indoor aspects related to the possible presence of fungi (visible filtration, fungi, mould odour, etc.).¹⁶

Antigens were obtained from Casa Alk-Abelló, Madrid, Spain. Skin tests were performed with the prick test.¹⁷ Sensitivity was tested to *Alternaria alternata*, *Neurospora sitophila*, *Aspergillus* mix (*amstelodami*, *fumigatus*, *niger* and *terreus*), *Penicillium* mix (*brevicompactum*, *expansum*, *notatum* and *roqueforti*), *Cladosporium* mix (*fulvum* and *herbarum*), *Chaetomium globosum*, *Curvularia spicifera*, *Fusarium moniliforme*, *Mucor mucedo*, *Rhizopus nigricans*, *Stemphylium botryosum* and *Candida albicans*.

Persistent allergic rhinitis was realised according to the WHO classification.¹⁸

Sera obtained from patients and controls were stored at –20°C until IgE analysis was performed. Specific serum IgE levels were determined by ultra RAST (Cypress Diagnostics, Langdorp, Belgium) radioimmunoassay, as much in the Control Group as in the Experimental Group to *A. alternata*, *N. sitophila*, *Aspergillus* mix, *Penicillium* mix, *Cl. herbarum* and *Ca. albicans* antigens.¹⁹

Air sample capture

Air samples were captured in July 2002, a rainy period of increased growth of fungi and most likely higher airborne fungal concentration. To measure and identify airborne fungi, two methods were used: the 'open Petri dishes' or sedimentation in agar Sabouraud medium (Difco, Detroit, MI, USA) with chloramphenicol (250 mg l⁻¹)²⁰ and RCS Plus (aeroscope), using agar strips (Biotest Hycon®, Dreieich, Germany).²⁰ Genera and species identification was carried out using traditional methods. Exposure limits to airborne fungi are currently not available.^{6,21} Interpretation of results was done according to Platt *et al.* classification system^{21,22} (Table 1).

Statistical analysis

Measurements from patients and controls were pooled for data analysis. Statistical analysis was performed using Student's *t*-test for unpaired data and Fischer's exact probability test. The limit for significance was taken as *P* < 0.05 (two-tailed).

Results

The most relevant results are assembled in Table 2 as frequencies of positive prick test, RAST to fungi and indoor/outdoor airborne fungal concentration at patient's domestic dwellings. The three most frequently identified fungal genera indoors and outdoors were *Cladosporium* spp., *Cephalosporium* spp. and *Aspergillus* spp. (Table 3). It is important to mention that prick test and RAST of some fungi held no relation whatsoever to airborne fungal concentrations in domestic dwellings.

Table 2 Frequencies (%) of positive prick test and specific serum IgE (RAST) to fungi in the Experimental Group ($n = 10$)

PRICK	
<i>Neurospora sitophila</i> *	40
<i>Candida albicans</i> *	30
<i>Aspergillus</i> * mix	30
<i>Alternaria alternata</i> *	30
<i>Cladosporium herbarum</i> and <i>Cladosporium fulvum</i> *	20
<i>Penicillium</i> * mix	10
RAST	
<i>Alternaria alternata</i> *	70
<i>Neurospora sitophila</i> *	50
<i>Candida albicans</i> *	40
<i>Penicillium</i> * mix	30
<i>Aspergillus</i> * mix	10
<i>Cladosporium herbarum</i> *	0

*Fungal antigens used in prick test and specific serum IgE.

Table 3 Frequencies (%) of different fungal genera identified by air samples by RCS Plus (captured indoors and outdoors)

Indoors	
<i>Cladosporium spp.</i> *	100
<i>Cephalosporium spp.</i>	70
<i>Aspergillus spp.</i> *	70
<i>Fusarium spp.</i> *	60
<i>Mycelia sterilia</i>	60
<i>Penicillium spp.</i> *	40
<i>Alternaria spp.</i> *	20
<i>Neurospora spp.</i> *	10
Outdoors	
<i>Cladosporium spp.</i> *	100
<i>Cephalosporium spp.</i>	80
<i>Aspergillus spp.</i> *	70
<i>Mycelia sterilia</i>	70
<i>Penicillium spp.</i> *	50
<i>Absidia spp.</i>	50
<i>Alternaria spp.</i> *	40

*Fungal antigens used in prick test and specific serum IgE.

All control subjects showed a negative prick test, but 20% of them had a positive RAST.

In this group dwellings, *Cladosporium* (100%), *Fusarium* (80%) and *Mycelia sterilia* (60%) were the three most frequently identified genera indoors and *Cladosporium* (100%), *Cephalosporium* (60%), *My. sterilia* (60%) and *Drechslera* (60%) outdoors.

Indoors a total of 36 different genera of fungi were identified in domestic dwellings of each group, outdoors 28 fungi in the control group and in the experimental group 30 different genera could be isolated and identified.

The average of the indoor count of fungal colonies in the control group, according to RCS Plus, was 182 CFU m⁻³ and in the experimental group 253 CFU m⁻³ (Table 4). Applying the Student's

Table 4 Indoor and outdoor fungi colonies counts (CFU m⁻³) in the dwellings of healthy subjects (Control Group) and patients (Experimental Group) by RCS Plus, using agar strips and aeroscope; Caracas, 8, 23 and 26 July 2002

	Fungi colonies in air (CFU m ⁻³)			
	Control group		Experimental group	
	Indoors	Outdoors	Indoors	Outdoors
1	175	960	190	nd
2	320	nd	130	nd
3	125	nd	330	110
4	115	220	120	155
5	125	170	380	245
6	75	75	255	170
7	220	140	235	245
8	305	250	525	690
9	225	190	255	145
10	135	125	105	225
Range	75–320	75–960	105–525	110–690
Average $\pm$ SE	182 $\pm$ 26	266 $\pm$ 101	253 $\pm$ 42	248 $\pm$ 66

unpaired *t*-test, there was not a statistically significant difference between both groups. Nevertheless, a tendency of higher indoor fungal concentrations in the experimental group than the control group was observed. In the case of the outdoor colonies count, the average was 266 CFU m⁻³ in the control group and 248 CFU m⁻³ in the experimental group (Table 4). Neither in this case was a statistically significant difference showed by Student's unpaired *t*-test between both groups. We found low (75 CFU m⁻³) and high (320 CFU m⁻³) indoor fungal levels in the control group. However, for the experimental group, the values oscillated between medium (105 CFU m⁻³) and high (525 CFU m⁻³) levels. On the other hand, outdoor fungal concentrations waved between low (75 CFU m⁻³) and high (960 CFU m⁻³) levels in the control group and between medium (110 CFU m⁻³) and high (690 CFU m⁻³) levels in the experimental group. We found a statistical significance ($P = 0.0486$) between indoor concentration fungal levels (CFU m⁻³; medium/high levels) and PAR clinical severity (mild and moderate/severe) in the experimental group by Fischer's exact probability test (Table 5).

Discussion

Fungi grow where there is food and continuous moisture. Most indoor environments contain airborne fungal spores and levels are normally influenced by outdoor concentrations. The most common species found in buildings belong to the genera *Cladosporium*,

Table 5 Relationship (%) between PAR of experimental group and CFU m⁻³ levels of indoor air by RCSPlus

CFU m ⁻³ level indoor	PAR	
	Mild/moderate	Severe
Moderate	30	40
High	20	10
Total	50	50

$P = 0.0486$.

Penicillium, *Alternaria* and *Aspergillus*. Condensation is the principal source of moisture that promotes growth of fungi on the internal surfaces of domestic housing. As well as other factors, like variation of light and temperature may affect spore formation and growth. The overall prevalence of fungi obtained in this survey showed that the three most frequent fungal genera outdoors, as well as indoors of domestic dwellings studied were *Cladosporium*, *Cephalosporium* and *Aspergillus*. Concerning the data collected outdoors, these are similar to those reported in temperate climate countries.^{10–12} Some studies in those countries associate exposure to allergens found in buildings indoors, as *Penicillium* spp., *Cladosporium* spp., *Aspergillus* spp. and others, with respiratory allergy in asthmatic patients.^{23,24}

The presence of fungal contamination is determined by visual inspection and sampling to identify and determine levels of airborne fungi. Indoor air samples must be compared with outdoor samples to determine if a problem exists. Where indoor concentrations are higher than outdoor, then indoor sources of fungi probably exists and should be controlled. The composition of fungal species should also be compared with outdoors and samples taken from other uncontaminated buildings.

The association between patients with respiratory allergy, a suggestive clinical history of exposure to spores and positive prick tests to moulds, may suggest that sources of fungi probably exist in patients' domestic dwellings and should be controlled. Nevertheless, no statistically significant association ($P = 0.1648$) between indoor fungal concentration of patients with respiratory allergy and prick test to moulds was found. This finding was similar to the results reported in Holland^{25,26} and Canada.²⁷ On the other hand, a statistically significant association between indoor fungal load and PAR severity levels was found.

According to some authors worldwide as well as in Venezuela, the fungal genera most frequently identified outdoors is *Cladosporium* spp.^{10–12} The three most

frequently identified genera in the air of the Experimental Group were *Cladosporium* spp., *Cephalosporium* spp. and *Aspergillus* spp. These results are similar to previous reports in Venezuela.¹⁰

Most of the surveys in template climate suggest that exposure to fungal allergen starts indoors.^{23,24} (Growth of *Penicillium* spp., *Cladosporium* spp., *Aspergillus* spp., *My. sterilia*^{25–28} in buildings is associated with an increased prevalence of respiratory symptoms and diseases, such as asthma and bronchitis. Although *Cladosporium* was the most frequent genera in all the studied environments, as much indoors as outdoors, it was not the most frequent one to whom patients or control subjects showed a higher sensitivity.

There is no exposure limits established for exposure to airborne fungi. Because of the variation in susceptibility of individuals to fungi and the diverse nature of fungi and their health effects, there are no 'safe' levels of exposure. However, it is generally believed that small amounts of mould growth, like those commonly found on shower walls, pose no immediate health risk to most individuals.

In addition, it is extremely difficult to coincide the sampling of fungal airborne spore load and the beginning of an allergic respiratory crisis and causally relate exposure to fungal spores and symptoms in the individual. As well as there is no consensus on the most clinically relevant methods for measuring personal exposure. Furthermore, multiple changing factors, such as climatologic variations and the time and site (residence, work, recreate place) of sampling will affect the existing fungal load.^{26–30}

In template climates home dwellings usually are closed, and heating systems and/or air conditioned control ambient air indoors. Therefore, occasionally higher spore concentrations may be found indoors than outdoors and the subjects affected use to remain long periods of time in these closed places, and an association may possibly be established. In the tropics, the natural ventilation of housing, without heating or cooling systems, allows a free fungal exchange between outdoors and indoors, which leads to maintain a balance of the frequency of airborne fungal species between indoor and outdoor and hence more difficult to support causality.

Immediate hypersensitivity to airborne fungal spores could not be established in this survey. In addition, many patients with respiratory allergy showed sensitivity to other allergens like pollens and dust mites, widely found in both outdoors and indoors of the studied dwellings, a fact which makes it difficult to attribute the clinical symptoms to fungal exposure alone.

On the other hand, the present study revealed a statistically significant association between indoor fungi CFU m⁻³ and PAR clinical severity. This suggests that fungal exposure could trigger allergic symptoms in patients with PAR and the clinical severity is strongly associated with indoor fungal concentration levels. Like Klánová K,²⁸ who conducted a similar study and found a positive association between indoor fungi levels and respiratory allergic clinical manifestations.

This was not true for outdoor fungal concentration levels in Caracas. Other authors in Caracas,¹⁰ observed a significant relationship between period of highest outdoor spores concentration and the symptoms severity in the asthmatic patients studied by them. These discrepancies may be because of the variability of the assessment of fungal exposure and changes in seasonal and weather patterns of fungal prevalence during the study.

Knowledge related to the relationship between spores inhaled and respiratory allergies induction or other health problems remains still inadequate and controversial in many aspects.²⁶

Although the results of this study reveal the limitations in assessing exposure-response relationships, it appears that fungal spores do play a role in clinical symptoms. More studies of fungal sensitivity should be conducted among susceptible individuals, and proper assessment of fungal prevalence of airborne spores may clarify the relationship between fungi and allergic symptoms in Venezuela.

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