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FUNGAL ALLERGIES IN KITCHEN ENVIRONMENT: A SHORT STUDY

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ABSTRACT
Some airborne fungi can cause allergy as well as severe infections, especially in poor indoor air quality environment, i.e. kitchen. The paper presents a pilot study taken to know if the fungal load in kitchen environments; especially in India, are correlated with allergic symptoms in persons who are in regular contact in such environment. Skin prick allergy tests were used for the evaluation of fungal allergy and sensitivity levels. The results showed that almost all isolated fungi triggered allergenic reaction in volunteers. *Aspergillus versicolor, Cladosporium herbarum, Cladosporium sp.* and *Penicillium sp.* were the highly allergenic fungi in kitchen environment of Jabalpur.

Key Words: Air quality, fungal load, fungal allergy, kitchen environment, skin prick test.

INTRODUCTION

The term allergy is defined as altered reactivity to exogenous antigens.[1] Allergic disease are known to occur due to the sustained over production of the Immunoglobulin E (IgE) and Immunoglobulin G (IgG) in response to the several environmental stimuli. IgG antibodies against microbes related to indoor dampness problems have been used as potential biomarkers of microbe exposure also in clinical investigations of asthma.[2] The major allergic manifestations induced by fungi are asthma, rhinitis, allergic bronchopulmonary mycoses, and hypersensitivity pneumonitis.[3]

Many fungal spores contain allergens which can trigger a range of respiratory symptoms in those susceptible, including sneezing, runny nose, mucous production, cough, congestion, sinusitis, earache, headache, wheezing, asthma and a range of bronchial symptoms and diseases. It is estimated that around 3-4% of the general population and 10% of atopic people get symptoms from fungal spores, while the majority of severe asthma sufferers are allergic to them. A number of fungal spore types share similar allergens which means that those who are allergic to moulds are likely to be sensitized to multiple types. Due to the fact that the different fungi vary in the release times of their spores, some people can therefore be affected for large parts of the year.[4]

Fungi can grow in damp or wet areas in the kitchen caused by water leaks, flooding, or high humidity that can result from everyday activities like cooking or washing. It can grow on wood, paper, fabrics, drywall and insulation. It can hide inside walls or above ceiling tiles. When fungus finds a damp place to grow, it can contribute to poor indoor air quality and health problems. People living in the kitchen and inside of stores with fungus and damp conditions commonly suffer with typical allergic symptoms.

Airborne microbial flora found commonly in kitchens, is some fungal genera and groups, *Penicillium*, yeasts, *Cladosporium*, and *Aspergillus*. Airborne fungi found in elevated concentrations in damp buildings are related to health problems.[5,6] *Botrytis cinerea* has been found in indoor air,[7] including in damp buildings,[8] and many people are allergic to the fungus *B. cinerea* in spite of its low airborne prevalence,[9] For example, 24% of 180 suspected mould-allergic patients[10] and 4% of 104 greenhouse workers [11] reacted positively to *Botrytis* in a skin prick test.

Since many clinical manifestations of allergy are mimicked by nonallergic mechanisms, it is usually necessary to use appropriate diagnostic procedures to ascertain whether the person has developed an IgE mediated immune response toward the incriminated allergen(s).[12] Such procedures primarily consist of skin tests, in which a small amount of allergen is applied on or injected into the skin. If the individual is sensitized, a local immediate reaction ensues, taking the form of a wheal (for IgE-mediated reactions), or swelling and redness occur after several hours (for delayed hypersensitivity reactions).

The present study investigated the fungal load in the kitchen environments of Jabalpur area for a period of one year. The fungal spores were cultured, and the fungi were isolated. These isolated fungi were extracted for the fungal allergens and were used for identification of fungal sensitivity using skin prick test. The objective of the study was to identify, if the fungi present in kitchen environment are able to trigger the allergic responses in those who are in regular contact with such fungal spores.

MATERIALS AND METHODS

Aerobiological Survey

Jabalpur (23°10'N, 79°57'E, MSL 402 m) is one of the major cities in Madhya Pradesh, India having more than 2.5 million population and spread over 75 km^2 area as urban populated area. The kitchen environments in Jabalpur were surveyed for aeromycoflora once a month in all seasons using Anderson two stage air samplers during 2015. Sabouraud Dextrose Agar was used for the capturing of airborne fungal spores.[13]

The fungal spores captured were cultured, isolated and identified. Identification of the fungi was carried out using reference slides and standard literature using books, monographs and reviews, published research papers as well as confirmation by respective experts in the field.[14,15]

Preparation of fungal antigens

Before starting the skin prick test on the selected individuals, the predominant fungi were grown in large amounts (1 L) in Sabouraud's dextrose broth in conical flasks for two months. The culture mass was harvested under aseptic conditions and was dried at 40°C for two days. The dried biomass was then sent to the ALCIT (India) Pvt. Ltd., New Delhi for the preparation of antigens. In total, fungal allergens from 16 fungal cultures were prepared and used.

Skin Prick Test

Before starting the skin prick tests, the selected individuals (volunteers) were interviewed for the symptoms of allergy as well as for their consent for participation in the study. The workers were interviewed for allergic symptoms they usually observe during different seasons as well as during their working hours in their respective kitchen. The individuals showing allergic signs and symptoms were shortlisted for skin prick test.

The response of fungal allergens on kitchen workers through skin prick test were undertaken at allergy clinic of Dr. Naveen Chawla, Jabalpur. Before prick, the patients were advised to avoid anti-histamine for 24 h and other sympathomimetic drugs like Ephedrine, Adrenaline, Isoprenaline, Ventolin, Animophylin for at least 8 h, i.e. last dose being taken at 12 mid night.

The sites selected for prick for performing antigen test were volar aspect of forearm and lateral aspect of upper arm, which are the usual test sites. The reactivity of skin to allergenic extract is not the same over the entire body. This variation from part to part may be due to the number of mast cells present per square cm of the epidermis in a given area. For antigen sensitivity test, phosphate buffered saline (PBS) acted as negative control, while Histamine acid (13 mg ml^{-1}) in PBS acted as positive control. The antigens (fungal allergens) were injected in 1:50 dilution in PBS. All the solutions were injected as a tiny drop by means of a draining needle under the supervision of medical practitioner.

The largest diameter of the wheal was measured in millimetre followed by measurement of the second diameter at right angle to the first. The mean of two diameters was recorded. Similar measurement was done for the Flare also. The length and number of pseudopodia were also recorded.

The diameter of the wheal as well as flare and pseudopodia were measured and recorded. Usually reactions of 2+ (wheel or flare of more than 4 mm) to 4+ (wheel or flare of more than 6 mm) were recorded as significant.

RESULTS

The fungal antigens procured from Alcit India Pvt. Ltd., New Delhi were used as such for the skin prick test. The antigens were selected from the major mycoflora observed during the survey work. Based on the survey findings, the antigens from the following fungal sources were used in the study:

1. Alternaria tenuis
2. Aspergillus flavus
3. Aspergillus fumigatus
4. Aspergillus niger
5. Aspergillus tamarii
6. Aspergillus versicolor
7. Candida albicans
8. Cladosporium cladospoiroides
9. Cladosporium herbarum
10. Cladosporium oxysporum
11. Cladosporium sp.
12. Curvularia lunata
13. Fusarium solani
14. Mucor mucedo
15. Penicillium sp.
16. Rhizopus nigricans

The fungal allergy tests using skin prick method were performed on normal female volunteers only. This was due to the fact that Indian household kitchens are usually occupied by the females, and hence they have far greater chance for having fungal allergy or sensitization.

The volunteers were interviewed for presence of fungal allergy symptoms i.e. cough, nasal blockage, breathlessness, sneezing, running nose, redness and watering of eyes and urticaria. The volunteers, who complained fungal allergy symptoms more frequently; were chosen for the fungal allergy tests at the allergy

clinic. The volunteers were subjected to skin prick tests with histamine as positive control and the fungal allergen preparations from 16 dominant fungi from kitchen environments of Jabalpur.

Table 1 shows that the response of volunteers to fungal allergens were in the range of +1 to +4. The response of +3 and +4 were categorized as severe allergic response, and the corresponding fungi was categorized as potent allergy causing fungi. The table shows that +4 response was observed in only four cases, and the responses were due to *Aspergillus versicolor, Cladosporium herbarum, Cladosporium sp.* and *Penicillium sp.* Out of these four cases, three were from the same volunteer, so it can be presumed that the response is more due to immunological response of the volunteer more than the allergen potency. Further, +3 response was observed with all of the fungal allergen in one or the other volunteer, showing that these fungi can be potentially allergenic.

Table 1: Results of skin prick test against the fungal allergens showing allergic response towards fungi isolated from kitchen environments of Jabalpur.

S. no.	Fungal Antigen	Patient's Response																		
		1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**
	+ve Control (mm)	5	4	8	6	6	6	5	5	7	6	6	4	5	5	4	5	4	5	6
1.	*Alternaria tenius*	+2	+2	+2	+3	+2	+3		+1	+3	+2	+2	+1		+3	+2	+2		+2	+3
2.	*Aspergillus flavus*	+2	+2	+2	+2		+3				+2	+3	+1		+2	+2	+3	+1	+2	
3.	*Aspergillus fumigatus*		+2	+2	+2		+3			+3	+2	+2	+1	+2	+2				+2	+2
4.	*Aspergillus niger*					+2	+1		+3				+3		+2	+2	+2		+3	+2
5.	*Aspergillus tamarii*		+2	+2	+2		+3	+3	+1	+2		+2		+1		+2	+2		+1	+1
6.	*Aspergillus versicolor*	+3			+2		+2	+3	+2	+4	+3	+2	+1		+1		+1		+1	
7.	*Candida albicans*				+3	+2	+3	+3	+3		+3					+2		+2	+2	+1
8.	*Cladosporium cladosporoides*	+2	+3		+3	+2	+2		+2	+3	+3	+2				+2	+1			
9.	*Cladosporium herbarum*	+4	+2	+3	+1	+2	+1		+3		+2	+1	+2	+2	+1		+1		+2	
10.	*Cladosporium oxysporum*		+2	+2	+2	+2			+2	+3	+3				+1	+1	+1			
11.	*Cladosporium sp.*				+2	+2	+2	+3	+2	+4	+2	+2			+2	+1	+1			+1
12.	*Curvularia lunata*		+2	+1	+3	+1	+3	+1	+1	+3			+1			+2	+2	+1	+1	
13.	*Fusarium solani*	+3		+1			+2	+3		+3	+2		+2		+2	+1	+1			+1
14.	*Mucor mucedo*			+1	+2	+3		+2	+2				+2			+2		+3	+2	+3
15.	*Penicillium sp.*				+2		+2	+3	+2	+4	+3			+3	+1	+3	+3		+3	+3
16.	*Rhizopus nigricans*			+1	+2		+3		+1	+3		+3	+1	+2						+3

Table 2 shows that there were 43 cases of +1 response, 85 cases of +2 response, 52 cases of +3 responses and 4 cases of +4 responses. It was clearly shown that the allergen caused moderate (+2) to severe (+3 and +4) allergic responses to the volunteers. A graphical representation of fungal allergy response is given in Fig 1. The yellow areas show moderate response while red and orange areas show severe allergic reactionas due to the fungal allergens in volunteers in Jabalpur city.

Table 2: Number of volunteers showing positive reactions during skin prick test upon exposure to fungal allergens from kitchen environments of Jabalpur.

S. No.	Fungal Antigen	No. of patients showing positive reaction			
		+1	**+2**	**+3**	**+4**
1.	*Alternaria tenuis*	2	9	5	-
2.	*Aspergillus flavus*	2	8	3	-
3.	*Aspergillus fumigatus*	2	9	2	-
4.	*Aspergillus niger*	1	5	3	-
5.	*Aspergillus tamarii*	3	7	2	-
6.	*Aspergillus versicolor*	4	3	3	1
7.	*Candida albicans*	1	4	5	-
8.	*Cladosporium cladospoiroids*	1	6	4	-
9.	*Cladosporium herbarum*	5	6	2	1
10.	*Cladosporium oxysporum*	3	5	3	-
11.	*Cladosporium sp.*	3	5	2	1
12.	*Curvularia lunata*	7	3	3	-
13.	*Fusarium solani*	4	4	3	-
14.	*Mucor mucedo*	1	6	3	-
15.	*Penicillium sp.*	1	3	5	1
16.	*Rhizopus nigricans*	3	2	4	-
	Total	**43**	**85**	**52**	**4**

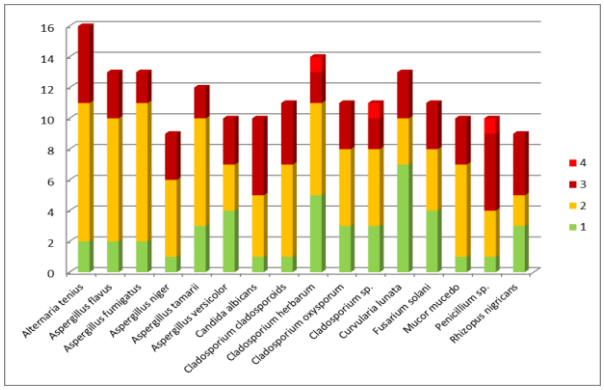

Fig 1: Allergic response to tested fungi in terms of skin prick test score by volunteers.

DISCUSSION

Various indoor allergens, including dust mites, animal dander, and mould have been reported to associate with allergic disease. Fungal spores and particles are widely distributed in outdoor and indoor environments and exposure to fungi has been shown to induce allergic disease and exacerbate symptoms in individuals sensitized to fungi.[16] Indoor dampness may favour microbial growth and cause greater exposure to fungi but also endotoxin and dust mites.[17] In India, the kitchen is a place where high level of dampness and poor air quality is often found. Usually, the Indian kitchens are clumsy, less aerated, less illuminated and over filled.

In some studies conducted with patients with asthma or allergic rhinitis showed no significant differences in the total spore count between cases and controls suggesting the type of fungi is more important than the total spore count. However, *Aspergillus*, *Penicillium* and basidiospores spp. significantly correlated with current asthma.[18] In a birth cohort study, exposure to specific fungal species in water-damaged buildings during infancy was associated with childhood asthma at 7 years.[19] Thus, it could be possible that specific fungal species are more important in developing allergy symptoms rather than the total fungal spore count. This indicates that fungal species may play a different role in allergic disease. There are accumulating evidences that precise recognition of species-specific sensitization to fungal allergens is important in the management of allergic disease.

The counting of fungal spores is itself not sufficient. It has been shown that fungal fragments and submicron particles of intra- and extracellular fungal structures contain detectable amounts of allergens and may therefore function as aeroallergen sources.[20] These particles are present in considerable larger quantities than spores, and respiratory deposition models indicate for some fungal species a much higher burden caused by fragments than by spores. Second, there is evidence that airborne allergen levels do not always correspond to spore or particle counts.[21] For example, it has been shown that depending on the developmental status, spores from *Alternaria alternata* release different amounts of the major allergen Alt a1.[22] The present study used Anderson sampler and sampling was done round the year in order to capture the fungal spores as well as any live fungal structure, i.e. fungal hypae and fragments so that to obtain the full aeromycoflora.

The first and most important step toward efficient allergen-specific forms of treatment is the proper diagnosis of fungal sensitization and the evaluation of the clinical relevance of the sensitizing allergens. Diagnosis of allergies, as routinely performed today, is a stepwise process, including skin tests and, if necessary, determination of total and allergen-specific IgE antibodies and other provocation tests.[23] The present study used skin prick tests in order to evaluate the allergenic potential of the isolated fungi.

To conclude, it was evident that during the skin prick tests, volunteers showed higher allergic response against the allergens from fungi, that were abundant most time of the year in kitchen environment of Jabalpur. An awareness related to fungi borne allergy and for the containment of aeromycoflora is highly needed.

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