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Skin Prick Test Reactivity to Common Airborne Pollens and Molds in Allergic Rhinitis Patients

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Summary

Allergic rhinitis is the single most common chronic allergic disease affecting an estimated four million people in Malaysia. House dust mites, grass pollens and fungal spores play has been identified to play a major role in the pathogenesis of allergic rhinitis. However, sensitization to pollen and spores in Malaysia is not well documented. On the basis of the results of an aerobiological survey of the common mold spores and pollens in the Klang Valley, twelve local extracts of molds and two local extracts of grass pollens were prepared by the Institute for Medical Research for this study. The study evaluated the prevalence of skin prick test (SPT) reactivity to the extracts of those airborne molds and pollens in allergic rhinitis patients in the Klang Valley. A total of 85 allergic rhinitis patients were recruited. All molds and grass pollens extracts tested, elicited positive response to SPT. Among the molds extracts, *Fusarium* was observed to have the highest prevalence of SPT reactivity (23.5%), followed by *Aspergillus flavum* (21.2%), *Dreselera orysae* (18.8%), *Alternaria sp* (17.6%), *Curvularis eragrostidis* (17.6%), *Penicillium oxa* (16.5%), *Pestolotriopsis guepini* (16.5%), *Rhizopphus arrhi* (16.5%), *Aspergillus nigus* (15.3%), *Penicillium chry* (12.9%), *Aspergillus fumigatus* (11.8%), and *Cladosporium sp* (4.7%). In the grass pollen, the SPT reactivity to *Ischaemum* and *Emilia is* 14.1% and 5.9% respectively. However, the prevalence of SPT reactivity was not influenced by the age, sex, ethnicity, symptomatology and concurrent allergic condition.
We have documented the prevalence of skin prick test reactivity to common molds and grass pollens in the Klang valley, which is comparable to the neighboring countries. Its prevalence in our allergic rhinitis patients suggests that it has a role in pathogenesis of allergic diseases. A larger representative sample involving multi-centric centers in Malaysia should be encouraged in the near future.

Key Words: Skin Prick Test Reactivity, Allergic Rhinitis, Mold, Pollen

Introduction

Allergic rhinitis is the most common allergic disorder and among the most common chronic diseases in the United States and other parts of the world including Malaysia. It is an Ig-E-mediated type-1 hypersensitivity disease of the mucus membranes of the nasal airways characterized by sneezing, itching, watery nasal discharge and sensation of nasal obstruction. It has two well-defined phases. An early phase reaction (EPR), clinically characterized by sneezing and nasal itchiness, developed 5 to 30 minutes post-allergen exposure involves the interaction between preformed mediators mainly histamine released by mast cells resulting vasodilatation, vascular leakage, increased mucus secretion and smooth muscle spasm such as bronchospasm. It usually becomes evident within 5 to 30 minutes after exposure to an allergen and subsides within an hour. A late-phase reaction (LPR) sets in 2 to 8 hours later without additional exposure to the antigen

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could lasted for several days, clinically characterized by nasal obstruction, with little sneezing and rhinorrhea.

Airborne pollen and spores allergens have long been implicated as one of the main causes of allergic respiratory problem in temperate country[1]. In Asia, particularly in Malaysia, studies on pollen or spore allergen are scarcely available. Endowed with year-round high temperature, high humidity level and without specific season provides excellent opportunity for fungal growth and dissemination.

Preliminary investigations have demonstrated that allergy to grass pollen and mold spores is present in Malaysia. Although, there are many species of molds and flowering plants, which are common in this region and potentially allergenic, only few of these local extracts of molds and pollen species are currently available as commercial allergenic solutions for use in SPT.

In this study, there were twelve common species of molds and two species of grass pollens obtained from the Klang Valley area, which were tested in the study population. Based on the frequency and abundance of each individual type found in our aerobiology survey and its potential allerginicity, they are considered the most relevant ones and potentially present in outdoor as well as indoor environment.

The skin prick test is used, as it is a recommended method by the European Academy of Allergology and Clinical Immunology, because of its safety and reliability[2]. It is also recommended in the United State as the most convenient and inexpensive screening method for the diagnosis of IgE-mediated allergic reaction[3]. Local study showed that the prevalence of SPT reactivity to common aeroallergens was found to be high among Malaysian asthmatics, particularly in those with an early age of onset and in those with co-existing rhinitis[4].

Materials and Methods

This is the prospective study where patients aged fifteen years and above suspected of having inhalant allergies with allergic rhinitis were randomly selected from the ENT clinic, HUKM. The diagnosis of allergic rhinitis was made by thorough history taking which includes patients symptoms (such as frequent sneezing, nasal itchiness, nasal obstruction and rhinorrhea), family history of allergic diseases and present of the other concomitant allergic condition (allergic conjunctivitis, asthma, allergic dermatitis) and clinical findings (inferior turbinate hypertrophy, pale overlying nasal mucosa, nasal crease). Participation was voluntary and the patients were given an "information sheet" to read and a "consent form" to complete. Patients 15 year-old and above with suggestive of allergic rhinitis and gave consent for the study were included.

Patients were excluded from study sample if they were found to be pregnant, on anti-histamine medication at least one week prior to the skin prick test or on other medication such as steroid, tricyclic anti-depression. The other exclusion criteria include severe dermatophagism, generalized eczema, history of severe allergic or anaphylaxis reaction and chronic debilitating diseases.

The objective of this study was to investigate the prevalence of skin prick test reactivity to local extracts of mold spores and grass pollens among allergic rhinitis patients in the Klang valley. Other objectives were to identify whether the general factors such as age, sex and race affect SPT reactivity. Also included in this study of allergic rhinitis patients are:

1. The influence of symptomatology (worst symptoms indoor or outdoor) to the prevalence of skin prick test reactivity to molds and grass pollens in allergic rhinitis patients.
2. The influence of associated allergic diseases to the prevalence of skin prick test reactivity to molds and grass pollens.

Study Approach

Culture of common outdoor and indoor molds was initiated and maintained for supply of the mold spores where as pollen bearing parts of the common grass were collected and used as a source of pollen. Molds and the grasses were identified by collaborators in the Botany Department, University Malaya. Extracts of the mold spores and pollen were prepared by the Division of Acarology, Institute for Medical Research. Sterility of the extracts were tested by the Division of Bacteriology, Instituted for Medical research. The extracts were standardized by prick testing on patients from the ENT Department, Hospital University Kebangsaan Malaysia (HUKM) who were suspected of inhalant allergies. Standardized extracts were tested on other patients to determine prevalence of allergies to those extracts.

Skin Prick Test Reactivity

The skin prick test was conducted at the ENT clinic, HUKM, by a trained paramedic under the supervision of a Medical Officer. All appropriate resuscitation equipment to treat adverse anaphylactic reactions was made available. A drop of allergen extract was applied on the skin of the forearm of each subject. A sterile lancet was used to lift the skin gently through the drop of allergen. Excess allergen was wiped off with sterile gauze. Histamine (as positive control) and diluent (as negative control) were included in the testing. After 15 minutes, the prick test site is examined. Cellophane tape was placed on each test site and the wheal traced using a permanent marker pen onto the tape. The presence of wheal 3 mm in diameter or larger than negative control was considered a positive reaction.

Commercial prick test solutions of mixed mold and grass pollen were included in the test to validate the presence of mold and/or pollen allergies. Statistical analysis of the results was carried out using Epi-info version 6 (CDC Atlanta). A p value of <0.05 was considered significant.

Results

Hundred and thirty-five patients with allergic rhinitis were randomly selected for skin prick test. Fifty patients were found to have negative reaction to both negative (normal saline) and positive control (histamine 1mg/ml) presumably suppressed by anti-histamine or other medications such as oral steroids were excluded from the study. Eventually 85 patients were included in this study.

The bulk of the patients were Malays (68.2%), followed by Chinese (17.6%) and Indian (14.2%). Females out-numbered males with the percentage of 57.6% and 42.4% respectively (Table I). Most of the patients were 45 years and below (85.9%). The mean and median age at presentation was 31.24 years and 30 years respectively.

The overall prevalence of SPT reactivity was highest to *Fusarium sp* (23.5%) and lowest to *Cladoprorium cladosporoides* (4.7%) for the molds species. In the grass pollen *Ischaemum* and *Emilia* registered 14.1% and 5.9% SPT reactivity respectively. (Table II)

Malays have higher reactivity to SPT in almost all of the molds species, followed by Chinese and Indian but statistically not significant. Among the Malay population, *Aspergillus flavus* have shown slightly higher percentage of reactivity to SPT as compare to *Fusarium spp.* The prevalence of skin prick test reactivity to grass pollen was also highest in Malays, followed by Chinese and Indian corresponding to the ethnic percentage of studied population. However, in molds and grass pollens, the differences in prevalence between races were also not statistically significant ($X < 3.84$, $p > 0.05$). (Table III)

Although the average of SPT reactivity was found highest in the age group 16 to 25 years, followed by 36 to 45 years, 26 to 35 years, and least in 56 years and above, the difference in prevalence in age group was not statistically significant in all molds and grass pollen species ($X < 3.84$, $p > 0.05$). (Table IV)

The percentage of those who claim that their symptoms were worse with indoors activities (50.6%) was greater as compared to those with outdoors activities (12.9%). However, 36.5 percent of patients could not determine whether their symptoms were worst indoors or outdoors (36.5%). Those with symptoms worse indoors had a higher average prevalence of SPT reactivity as compared to those with symptoms worst outdoors. However, this was not statistically significant in all molds and grass pollens ($X < 3.84$, $p > 0.05$). (Table V)

Associated allergic diseases (atopy) such as asthma, eczema or allergic conjunctivitis were also analyzed. Thirty-seven (43.5%) patients were found to have associated atopic conditions. On the other hand, 48.2% have no associated atopic conditions and 8.2% were uncertain. The prevalence of skin prick test reactivity to molds and pollens were found higher in patients with atopic conditions. However this was not statistically significant ($X < 3.83$, $p > 0.05$). (Table VI)

Table I: Distribution of allergic rhinitis patients according to ethnicity and sex

Ethnic	Male		Female		Total	
	n	%	n	%	n	%
Malay	20	34.5	38	65.5	58	68.2
Chinese	10	66.6	5	33.4	15	17.6
Indian	6	50	6	50	12	14.2
Total	36	42.4	49	57.6	85	100

Table II: Overall prevalence of SPT reactivity to molds and grass pollens in allergic rhinitis patients

ALLERGENS	Sample	
MOLDS	n	%
Alt.sp	15	17.6
Asp.fla	18	21.2
Asp.fum	10	11.8
Asp nig	13	15.3
Clad.c	4	4.7
Curv.era	15	17.6
Dres.ory	16	18.8
Fusa.sp	20	23.5
Pen.chry	11	12.9
Pen.oxa	14	16.5
Pest.gue	14	16.5
Rhiz.arrhi	14	16.5
GRASS POLLENS		
Ischaemum	12	14.1
Emilia	5	5.9

Table III: The prevalence of SPT reactivity of each species of molds and grass pollens according to ethnic group

ALLERGENS	Malay		Chinese		India	
MOLDS	n	%	n	%	n	%
Alt.sp	8	9.4	5	5.9	2	2.4
Asp.fla	11	12.9	5	5.9	2	2.4
Asp.fum	6	7	2	2.4	2	2.4
Asp nig	7	8.2	4	4.7	2	2.4
Clad.c	1	1.2	1	1.2	2	2.4
Curv.era	8	9.4	4	4.7	3	3.5
Dres.ory	9	10.5	3	3.5	4	4.7
Fusa.sp	10	11.8	6	7	4	4.7
Pen.chry	5	5.9	3	3.5	2	2.4
Pen.oxa	6	7	5	5.9	3	3.5
Pest.gue	8	9.4	4	4.7	2	2.4
Rhiz.arrhi	8	9.4	4	4.7	2	2.4
GRASS POLLENS						
Ischaemum	7	8.2	3	3.5	1	1.2
Emilia	3	3.5	2	2.4	0	0

Table VI: The prevalence of SPT reactivity to molds and grass pollens according to age group

ALLERGENS	16–25 years		26-35 years		36-45 years		46-55 years		>55 years	
MOLDS	n	%	n	%	n	%	n	%	n	%
Alt.sp	3	3.5	4	4.7	4	4.7	3	3.5	1	1.2
Asp.fla	4	4.7	5	5.9	4	4.7	4	4.7	1	1.2
Asp.fum	2	2.4	3	3.5	3	3.5	2	2.4	0	0
Asp nig	5	5.9	3	23.1	3	3.5	1	1.2	1	1.2
Clad.c	1	1.2	1	1.2	1	1.2	1	1.2	0	0
Curv.era	4	4.7	3	3.5	3	3.5	4	4.7	1	1.2
Dres.ory	5	5.9	4	4.7	3	3.5	3	3.5	1	1.2
Fusa.sp	7	8.2	3	3.5	5	5.9	2	2.4	3	3.5
Pen.chry	3	3.5	3	3.5	3	3.5	1	1.2	1	1.2
Pen.oxa	4	4.7	3	3.5	4	4.7	3	3.5	0	0
Pest.gue	4	4.7	4	4.7	3	3.5	2	2.4	1	1.2
Rhiz.arr	3	3.5	4	4.7	3	3.5	3	35	1	1.2
G/POLLENS										
Ischaem	3	3.5	3	3.5	3	3.5	2	2.4	1	1.2
Emilia	1	1.2	2	2.4	1	1.2	1	1.2	0	0

Table V: The prevalence of SPT reactivity to molds and grass pollens according to type of activity

ALLERGENS	Indoors		Outdoors		Unsure	
MOLDS	n	%	n	%	n	%
Alt.sp	6	7	3	3.5	6	7
Asp.fla	9	10.5	4	4.7	5	5.9
Asp.fum	3	3.5	6	7	1	1.2
Asp nig	5	5.9	5	5.9	3	3.5
Clad.c	3	3.5	1	1.2	0	0
Curv.era	7	8.2	2	2.4	6	7
Dres.ory	7	8.2	2	2.4	7	8.2
Fusa.sp	9	10.5	5	5.9	6	7
Pen.chry	5	5.9	3	3.5	3	3.5
Pen.oxa	7	8.2	3	3.5	4	4.7
Pest.gue	6	7	4	4.7	4	4.7
Rhiz.arr	7	8.2	3	3.5	4	4.7
G/POLLENS						
Ischaem	4	4.7	4	4.7	4	4.7
emilia	2	2.4	1	1.2	1	1.2

Table VI: The prevalence of SPT reactivity to molds and grass pollens according to associated allergic diseases (atopy)

ALLERGENS	(+) allergic disease		(-) allergic disease	
MOLDS	n	%	n	%
Alt.sp	7	8.2	8	9.4
Asp.fla	10	11.8	8	9.4
Asp.fum	6	7	4	4.7
Asp nig	9	10.5	4	4.7
Clad.c	3	3.5	1	1.2
Curv.era	8	9.4	7	8.2
Dres.ory	8	9.4	7	8.2
Fusa.sp	13	15.3	7	8.2
Pen.chry	7	8.2	4	4.7
Pen.oxa	8	9.4	6	7
Pest.gue	8	9.4	6	7
Rhiz.arr	9	10.5	5	5.9
G/POLLENS				
Ischaem	6	7	6	7
Emilia	3	3.5	2	2.4

Discussion

The prevalence of SPT reactivity to molds and grass pollens in this study ranges from 4.7% to 23.5%. In fact, most of the molds and grass pollens gave a prevalence of more than 15% SPT reactivity, comparable to other studies in Southeast Asia such as Thailand and Singapore. *Fusarium sp*, with prevalence of 23.5% to SPT reactivity, is the highest among mold species whereas *Ischaemum* is higher as compared to *Emilia* among the grass pollens. There are no previous studies on both grass pollens in this region.

Moisture or humidity is important to support fungal growth as fungal growth can be reduced or stopped by controlling moisture[5]. South-East Asia region with high in temperature and humidity favour fungal growth and dissemination. Thus, the prevalence of SPT reactivity is expected to be higher compared to European countries.

The prevalence of SPT reactivity to molds and grass pollens in Thailand and Singapore are comparable to our study. In Thailand, the prevalence of SPT reactivity to common grasses and mold ranges from 11% to 26%[5]. On the other hand, in Singapore the prevalence of SPT reactivity was relatively high especially to *Curvularia sp* (26%-32%) and *Drechslera*-like spores (31%)[6]. They also found that positive responses to these extracts correlated with total serum IgE levels of the subjects and were significantly associated with the presence of atopic disease. In Italy, the prevalence varied considerably among the different centers from a minimum of 1.8% (Turin) and 1.9% (Genoa) to a maximum of 24% (Cagliari)[7]. According to the author, the variation in SPT results could be linked to the purity of the diagnostic extract employed and the weather conditions affecting the region. Across Europe, the highest percentage of positive subjects was found in Spain (20%); lower percentages were found in Germany, Greece and Austria, with the lowest percentage in Portugal (3%)[8]. As mentioned, *Alternaria* and *Cladosporium* were the most common species studied.

In this study, *Aspergillus, Alternaria, Curvularia, Dreselera, Pestolotriopsis guepini and Rhizopus* were among the mold species that gave a markedly high prevalence in Malays as compared to other ethnics. Both the grass pollens also showed higher prevalence in Malay patients However, both findings were not statistically significant.

It is known that the allergic diseases usually present early in life as compared to chronic diseases like diabetes, hypertension etc. According to age distribution, majority of the samples are 45 years and below (83.3%). The remaining are 46 years and above

(16.7%). The prevalence of SPT reactivity to molds and grass pollen were generally higher in age of 45 and below but this was not statistically significant.

Sixty-four (50.4%) allergic rhinitis patients had their symptoms worst indoor while 16 (12.6%) patients had worst symptoms outdoor and 47 (37%) were unsure. This is not surprising because indoor molds can be found outdoors and vice versa[5]. The SPT to molds and grasses showed that those who have worst symptoms indoor have higher prevalence than those with worst symptoms outdoor except for *Aspergillus fumigatus*, but it was not statistically significant.

Beside allergic rhinitis, there are several other atopic diseases such as asthma, skin diseases (eczema and allergic dermatitis) and allergic conjunctivitis. In this study, 55 patients (43.3%) were found to have at least one other atopic condition and 72 patients (56.7%) were either not associated with other allergic diseases or were unsure of status.

Although patients with other atopic conditions were found to have higher prevalence of SPT reactivity as compared to those without except for *Alternaria* and *Emilia*, which have higher prevalence to the contrary, which was not statistically significant.

Also observed in this study was that those mold-allergy individuals often showed reactivity to more than one fungus. This may be explained by the possibility of the presence of the cross-reactivity proteins in different fungi. Many studies revealed that patients sensitive to one fungus show positive skin reactions and raised serum IgE to several other fungal allergens[10,11,12] but this can only be proven by doing laboratory test like imunoblot and enzyme-linked immunoabsorbent assay (ELISA) test to the patient's serum, which was not done to our study population.

Conclusion

This study revealed that the prevalence of SPT reactivity to mold is highest for Fusarium (23.5%), followed by *Aspergillus flavum* (21.2%), *Dreselera orysae* (18.8%), *Alternaria sp* (17.6%), *Curvularis eragrostidis* (17.6%), *Penicillium oxa* (16.5%), *Pestolotriopsis guepini* (16.5%), *Rhizopphus arrhi* (16.5%), *Aspergillus nigus* (15.3%), etc, whereas the prevalence of SPT reactivity to Ischaemum and Emilia pollens was 14.1% and 5.9% respectively. This data was comparable to neighboring countries like Thailand and Singapore.

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