

Original article

Evaluation of the allergenicity of tropical pollen and airborne spores in Singapore

Background: Sensitization to pollen and spores of the Southeast Asian tropical region is not well documented. This study evaluated the allergenicity of the tropical airspora in Singapore.

Methods: On the basis of the results of an aerobiologic survey of the airspora profile of Singapore, crude extracts of 23 main spore (fungal and fern) and pollen types were prepared. A total of 231 patients with asthma and/or allergic rhinitis and 76 healthy controls were evaluated by skin prick test (SPT). Total and specific IgE levels were also quantified by the fluorescence allergosorbent test (FAST).

Results: All 23 allergenic extracts tested elicited positive SPT responses. Among the patients with atopic diseases, extracts of oil-palm pollen (*Elaeis guineensis*) were observed to have the highest frequency of positive reactions (40%), followed by extracts of resam-fern spores (*Dicranopteris linearis*) (34%) and sea-teak pollen (*Podocarpus polystachyus*) (33.8%). Fungal spores with the highest SPT responses were *Curvularia* spp. (26–32%) and *Drechslera*-like spores (31%).

Positive responses to these extracts correlated with total serum IgE levels of the subjects and were significantly associated with the presence of atopic disease.

Conclusions: We have documented sensitization to tropical pollen and spores in our population. Its association with atopy suggests that it has a role in allergic diseases in the tropics.

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Allergic respiratory diseases such as asthma and rhinoconjunctivitis are common disorders (1). Asthma is a common cause of childhood morbidity in Singapore and is a growing concern there (2, 3). Rhinoconjunctivitis is also a major cause of morbidity. The prevalence of rhinitis is reported to be 44% among children (2) and 25–33% in adults (4, 5).

Airborne pollen and spore allergens have been implicated as one of the main causes of allergic respiratory problems in temperate countries (6, 7). In tropical Asia, little information on pollen or spore allergens is available. Aerobiologic surveys in Singapore show an abundance of outdoor airborne spores and pollen year round (8). Recent studies have also suggested that the trends for acute asthma exacerbation were associated with variations in the local airspora profile (9, 10). In this study, we set out to evaluate the allergenicity of the airspora of Singapore.

Material and methods

Collection and culture of raw material for extract preparation

According to the results of the aerobiologic survey of the Singapore outdoor environment, the most abundant spores/pollen types were selected for crude extract

preparation (Table 1). The spores of six fern species (pteridophytes) were collected from various sites in Singapore. The mature fertile fronds with sporangia were collected and kept dry at room temperature for 2–3 days. Spores were collected by scraping the sporangia with a toothbrush and were passed through an 80-mesh sieve to remove extraneous plant debris. The pollen of five seed plants was collected from mature inflorescence. The pollen was readily obtained by agitating the mature inflorescence and allowing the released pollen to fall into a large container lined with clean, smooth-glazed paper. The harvested pollen was further separated from extraneous plant and inflorescence debris by sequential sieving. The purity of the collections was confirmed under a light microscope. The spores and pollen were frozen at -80°C until further use.

Six genera of fungal spores were identified for evaluation of allergenicity. The identities of these spores were further confirmed to species level through sporulating cultures (Table 1). Their identities were confirmed by the International Mycological Institute (IMI), UK. The 12 fungal isolates were mass cultured according to their predetermined optimal culture

Table 1. Spore/pollen types selected for crude extract preparation

Botanic name	Common name	Spore type
<i>Acacia auriculiformis</i> A. Cunn. ex Benth.	Common acacia	Tree pollen
<i>Asplenium nidus</i> L.	Bird's nest fern	Fern spore
<i>Bipolaris</i>	—	Fungal spore
<i>Casuarina equisetifolia</i>	Rhu	Tree pollen
<i>Cladosporium</i>	—	Fungal spore
<i>Corynespora</i>	—	Fungal spore
<i>Curvularia brachyspora</i> Boedijn	—	Fungal spore
<i>C. fallax</i> Boedijn	—	Fungal spore
<i>C. inequalis</i> (Shear) Boedijn	—	Fungal spore
<i>C. lunata</i> (Wakker) Boedijn	—	Fungal spore
<i>C. pallescens</i> Boedijn	—	Fungal spore
<i>Dicranopteris curranii</i> Copel.	Resam fern	Fern spore
<i>Dicranopteris linearis</i> (Burm.f.) Underw.	Resam fern	Fern spore
<i>Didymosphaeria</i>	—	Fungal spore
<i>Elaeis guineensis</i> Jacq.	Oil palm	Palm pollen
<i>Exserohilum</i>	—	Fungal spore
<i>Kyllingia polyphylla</i> Willd. ex Kernth	Greater kyllinga	Sedge pollen
<i>Nephrolepis auriculata</i> (L.) Trimen	Ladder fern	Fern spore
<i>Pithomyces</i>	—	Fungal spore
<i>Podocarpus polystachyus</i> R.Br. ex Endl.	Sea teak	Tree pollen
<i>Pteridium aquilinum</i> (L.) Kuhn	Bracken fern	Fern spore
<i>Stenochlaena palustris</i> (Burm.f.) Bedd.	Climbing swamp fern	Fern spore
<i>Tetraploa</i>	—	Fungal spore

conditions. Various media and combinations of culture conditions were tested initially to determine the optimal conditions which induced sporulation of the 12 fungal isolates. The media tested were Czapek's agar (M312), Czapek Dox agar (modified), malt extract agar (Blake Slee's formula) (M325), oatmeal agar, potato dextrose agar, potato carrot agar, rabbit food agar (M340), sporulation agar (M5), and V-8 Juice agar (M343). The media were tested in combinations with or without black light, with black light and with or without rice leaves, and without black light and with or without rice leaves (with some exceptions, no rice leaves were used in combination with some media). The rice leaves used either were a single piece placed on the agar surface as for most of the media or were macerated and included in the media for potato carrot agar and potato dextrose agar. All media and rice leaves were sterilized by autoclaving at 1.2 kfg cm⁻² pressure for 20 min.

The optimal culture conditions for the 12 fungal isolates were as follows:

- 1) potato dextrose agar (PDA) for *Curvularia fallax*, *C. inequalis*, *C. pallescens*, *C. brachyspora*, *C. lunata*, *Bipolaris* sp., *Exserohilum rostratum*, *Pithomyces maydis*, and *Cladosporium cladosporioides* with 9 h black light per day at 25°C
- 2) potato dextrose agar with 5 g rice leaves per liter medium for *Corynespora cassicola* and 9 h black light per day at 25°C
- 3) potato dextrose agar with strips of rice leaves for *Tetraploa aristata* and 9 h black light per day at 25°C
- 4) oatmeal agar (OMA) for *Didymosphaeria donacina* incubated at 25°C.

Cultures were incubated at 25°C, and sporulation was induced with 9 h black light per day (with the exception of *D. donacina*). The fungal isolates were cultured until sporulation occurred (2–3 weeks depending on the spore type). Sporulating cultures were then collected with a custom-made vacuum pump device and stored at –80°C until further use.

Extract preparation

The crude extracts were prepared in Coca's solution according to a modified procedure by Sheldon et al. (11). For each extract, five different batches of collections or cultures of raw material were used to minimize variations in the extracts due to source material. The spores/pollen were defatted with anhydrous ethyl ether (×3) and then extracted in solution (100 mM sodium chloride, 50 mM sodium bicarbonate, and 0.02% phenol) at a ratio of 1.0 g of spores/pollen to 10 ml of extraction fluid. The spores/pollen were homogenized in solution with a mortar and pestle, and rotated for 24 h at 4°C. The extracts were then centrifuged, sterilized by filtration, and then diluted 1:1 with glycerin and stored at 4°C. The protein concentration of extracts was assayed by the method of Bradford (12) with the Bio-Rad protein dye-binding assay, and standardized by protein concentration.

Study population

A total of 307 individuals (186 males and 121 females) were evaluated. They included 131 outpatients recruited from the pediatric asthma clinic of the National University Hospital (further divided into two age-groups; 2–5-year-olds [*n*=47] and 6–14-year-olds [*n*=84]) and 176 volunteers (>14 years old) (100 had at least allergic rhinitis and/or asthma, and there were 76 healthy controls). All subjects were residents of Singapore. Consent was obtained from all participants and/or their guardians. The presence of atopic disease (atopic eczema, allergic rhinitis, and bronchial asthma) was based on clinical history. Persons with atopic eczema were defined as those currently suffering from or having a history of chronic or relapsing pruritic rash in the flexural areas of limbs or around the neck. Asthma was determined by the report of a physician's diagnosis of asthma or complaints of frequent wheezing in the past 12 months. Allergic rhinitis was defined as frequent paroxysms of sneezing, nasal pruritus, congestion, or profuse watery nasal discharge. It was also ascertained that they were asymptomatic and not taking any medication at the point of evaluation. The subjects were categorized as patients with at least one of the reported allergic disorders, and as healthy controls when all three conditions were ruled out. Table 2 summarizes the demographic profile of the individuals evaluated according to the presence of an atopic disease and age group.

Serum samples of 176 patients (92 children and 84 adults) and 24 healthy controls were collected and screened for total and specific IgE. Serum samples were stored at -20°C until assayed.

Skin prick tests (SPT)

A reaction of greater than 3×3 mm wheal diameter 20 min after pricking was regarded as a positive prick. This criterion was based on the description by Brown et al. (13) that prick reactions of 3–5 mm or more are very likely to be due to circulating specific IgE. Glycerol buffer (50% glycerol) and histamine (1 mg/ml) were included as negative and positive controls, respectively. Buffer controls of the media and/or rice leaves used for the culture of fungal spores were also prepared and included in the panel.

Quantification of specific IgE levels via fluorescence allergosorbent test (FAST)

Total and specific serum IgE antibodies were measured by quantitative FAST (BioWhittaker, USA), as described previously (14). Custom-coated wells were prepared for local pollen and spores (10–25 $\mu\text{g}/\text{well}$), according to the manufacturer's instructions. The optimal coating concentrations for each spore/pollen type were determined by a series of titrations, such that sera of skin-prick-negative healthy controls showed readings of <0.2 IU/ml, and sera of those with allergen-specific SPT-positive responses standardized by skin end-point titration showed readings greater than 0.35 IU/ml. These were further standardized by FAST inhibition. The intra-assay variations were less than 5% for all assays, and the interassay variations were 10.8–18.7% (depending on the spore/pollen type).

Statistical analysis

Analysis was done by the statistical package SAS v6.08 for Windows (15). Data were analyzed by the chi-square test or Fisher's exact test to compare the sensitization rates between the patient groups and healthy controls. Comparison between specific IgE levels was made with Spearman's rank correlation test. Comparisons between normalized mean levels of total or specific IgE of two groups were made by the unpaired *t*-test. However, analysis of variance (ANOVA) with Duncan's new multiple range test (DNMRT) was used for multiple comparison of the means.

Results

SPT responses

Table 3 shows SPT responses of the subjects. Sensitization to a commercial preparation of the dust mite *Dermatophagoides pteronyssinus* (10 000 AU/ml; Greer Laboratories, Lenoir, NC, USA) was included for comparative purposes. In most of the extracts tested, the sensitization rates of adult "atopic" patients were significantly higher than healthy controls, with the exception of *Cladosporium*. The sensitization rates among young asthmatic children (3–5 years) for most of the extracts were not significantly different from healthy adult controls. Among the patients with atopic diseases, oil-palm pollen (*Elaeis guineensis*) was the pollen/spore with the highest frequency of positive reactions (39.8%). This was followed by extracts of resam-fern spore (*Dicranopteris linearis*) (34.2%) and sea-teak pollen (*Podocarpus polystachyus*) (33.8%). *Curvularia* spp. (26.0–31.6%) and *Drechslera*-like spores (30.7% except for *Corynespora*) had the highest positive SPT responses among the fungi. There were no

Table 2. Demographic profile of individuals evaluated

	Healthy controls*	Asthma and/or allergic rhinitis patients*		
		3–5-year-olds	6–14-year-olds	> 14 years old
Mean age (years), range	<i>n</i> = 76 22.5 (17–47)	<i>n</i> = 47 4.3 (3–5)	<i>n</i> = 84 8.3 (6–14)	<i>n</i> = 100 21.7 (15–49)
Sex				
M	40 (52.6%)†	27 (57.4%)†	50 (59.5%)	63 (63.0%)†
F	36 (47.4%)	20 (42.6%)	34 (40.5%)	37 (37.0%)
Ethnic group				
Chinese	67 (88.2%)	30 (63.8%)	57 (67.9%)	92 (92.0%)
Malay	6 (7.9%)	9 (19.1%)	13 (15.5%)	6 (6.0%)
Indian	3 (3.9%)	4 (8.5%)	8 (9.5%)	2 (2.0%)
Others	0 (0.0%)	4 (8.5%)	6 (7.1%)	0 (0.0%)
Atopic condition‡				
Asthma	—	47 (100.0%)	84 (100%)	47 (47.0%)
Allergic rhinitis	—	17 (36.2%)	50 (59.5%)	87 (87.0%)
Eczema	—	27 (57.4%)	38 (45.2%)	24 (24.0%)

*Only adult healthy control volunteers were included in this study. Patients with atopic diseases were divided into three groups according to their age groups: 3–5 years old, 6–14 years old, and > 14 years old.

†Values in parenthesis are percentages unless otherwise stated.

‡All children in this study were recruited from the asthma clinic and thus had asthma. However, they may have had other atopic conditions concurrently.

Table 3. Frequency of skin test sensitivity to allergens tested

		Asthma and/or allergic rhinitis patients			
	Healthy controls (<i>n</i> =76)	All (<i>n</i> =231)	3–5 years (<i>n</i> =47)	6–14 years (<i>n</i> =84)	> 14 years (<i>n</i> =100)
	Percentage skin test positive (%)				
Dust mite (for comparison)					
<i>Dermatophagoides pteronyssinus</i>	26 (34.2%)	220 (95.2%)	42 (89.4)***	79 (94%)***	99 (99%)***
Single species spore types					
<i>Cladosporium</i>	7 (9.2)	38 (16.5)	3 (6.4)	15 (7.9)	20 (20.0)
<i>Didymosphaeria</i>	9 (11.8)	63 (27.3)	7 (14.9)	20 (23.8)	36 (36.0)***
<i>Pithomyces</i>	7 (9.2)	46 (19.9)	7 (14.9)	13 (15.5)	26 (26.0)**
<i>Tetraploa</i>	5 (6.6)	37 (16.0)	2 (4.3)	10 (11.9)	25 (25.0)**
<i>Curvularia</i> spp. spores					
<i>C. brachyspora</i>	8 (10.5)	64 (27.7)	8 (17.0)	20 (23.8)*	36 (36.0)***
<i>C. fallax</i>	6 (7.9)	64 (27.7)	10 (21.3)	24 (28.6)**	30 (30.0)***
<i>C. inequalis</i>	0 (0.0)	60 (26.0)	9 (19.1)***	18 (21.4)***	33 (33.0)***
<i>C. lunata</i>	5 (6.6)	60 (26.0)	10 (21.3)*	20 (23.8)**	30 (30.0)***
<i>C. pallescens</i>	2 (2.6)	73 (31.6)	10 (21.3)***	22 (26.2)***	41 (41.0)***
<i>Drechslera</i> -like spores					
<i>Bipolaris</i>	5 (6.6)	71 (30.7)	8 (17.0)	30 (35.7)***	33 (33.0)***
<i>Corynespora</i>	6 (7.9)	71 (30.7)	8 (17.0)	27 (32.1)***	36 (36.0)***
<i>Exserohilum</i>	6 (7.9)	41 (17.7)	6 (12.8)	13 (15.5)	22 (22.0)*
Fern spores					
<i>Asplenium nidus</i>	3 (3.9)	59 (25.5)	6 (12.8)	24 (28.6)***	29 (29.0)***
<i>Dicranopteris curranii</i>	7 (9.2)	71 (30.7)	11 (23.4)	20 (23.8)*	40 (40.0)***
<i>Dicranopteris linearis</i>	6 (7.9)	79 (34.2)	11 (23.4)*	24 (28.6)**	44 (44.0)***
<i>Nephrolepis auriculata</i>	7 (9.2)	68 (29.4)	7 (14.9)	27 (32.1)***	34 (34.0)***
<i>Stenochlaena palustris</i>	9 (11.8)	50 (21.6)	5 (10.6)	7 (8.3)	38 (38.0)***
<i>Pteridium aquilinum</i>	6 (7.9)	47 (20.3)	5 (10.6)	13 (15.5)	29 (29.0)**
Pollen					
<i>Acacia auriculiformis</i>	6 (7.9)	64 (27.7)	5 (10.6)	23 (27.4)**	36 (36.0)***
<i>Casuarina equisetifolia</i>	5 (6.6)	64 (27.7)	5 (10.6)	25 (29.8)***	34 (34.0)***
<i>Kyllingia polyphylla</i>	7 (9.2)	58 (25.1)	10 (21.3)	16 (19.0)	32 (32.0)***
<i>Elaeis guineensis</i>	9 (11.8)	92 (39.8)	12 (25.5)	32 (38.1)***	48 (48.0)***
<i>Podocarpus polystachyus</i>	6 (7.9)	78 (33.8)	9 (19.1)	29 (34.5)***	40 (40.0)***

Chi-square test or Fisher's exact test was performed to compare individual patient groups with healthy control group. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

subjects monosensitized to pollen or spores. All pollen- and spore-sensitized individuals were also sensitized to dust mites.

Patients with atopic diseases had a larger number of SPT-positive responses to these allergens than healthy

controls (Table 4). In addition, the number of positive responses showed an increasing trend with the age of the subjects with atopic diseases, except for the fungal spores, which did not reach statistical significance. The geometric mean total serum IgE levels also correlated

Table 4. Number of skin-test-positive reactions and total IgE levels

		Asthma and/or allergic rhinitis patients			
		Healthy controls (n=76)	3–5 years (n=47)	6–14 years (n=84)	> 14 years (n=100)
Total IgE (IU/ml)		103.1 IU/ml	1025.6 IU/ml	1657.2 IU/ml	1125.0 IU/ml
(SD)†		(20.4–378.8)†	(342.0–4058.9)†	(578.1–5062.5)†	(265.8–3910.5)†
Mite-allergen sensitivity	% prick positive	26 (34.2%)	42 (89.4%)	79 (94.0%)	99 (99.0%)
Fungal spore sensitivity	≥ 7 reactions	0 (0.0%)	2 (4.8%)	11 (13.1%)	17 (17.0%)
(no. of prick positive)	≥ 5 reactions	3 (3.9%)	5 (10.6%)	19 (22.6%)	30 (30.0%)
	≥ 3 reactions	9 (11.8%)	10 (21.3%)	30 (35.7%)	56 (56.0%)
	At least 1	29 (38.2%)	34 (72.3%)	69 (82.1%)	88 (88.0%)
Fern-spore sensitivity	≥ 5 reactions	0 (0.0%)	2 (4.3%)	6 (7.1%)	13 (13.0%)
(no. of prick positive)	≥ 3 reactions	5 (6.6%)	7 (14.9%)	18 (21.4%)	49 (49.0%)
	At least 1	19 (25.0%)	27 (57.4%)	49 (58.3%)	74 (74.0%)
Pollen sensitivity	Reacted to all 5	0 (0.0%)	1 (2.1%)	5 (6.0%)	8 (8.0%)
(no. of prick positive)	≥ 3 reactions	3 (3.9%)	4 (8.5%)	19 (22.6%)	44 (44.0%)
	At least 1	20 (26.3%)	20 (42.6%)	57 (67.9%)	80 (80.0%)

†Serum samples of only 176 patients (23 children aged 3–5 years, 69 children aged 6–14 years, and 84 adults) and 24 healthy controls were collected and screened for total IgE.

Table 5. Serum total IgE levels and number of positive skin prick reactions to local airspora allergens

No. of positive reactions*	No. of individuals	Geometric mean total IgE (IU/ml) (SD)†
0	21	91.23 ^b (22.91–342.74)
1–3	54	610.36 ^{ab} (125.02–2956.10)
4–6	46	1021.83 ^{ab} (264.35–3912.98)
>6	79	1465.72 ^a (432.08–4966.35)

*Serum samples of only 176 patients (23 children aged 3–5 years, 69 children aged 6–14 years, and 84 adults) and 24 healthy controls were collected and screened for total IgE.

†Means with same letter are not significantly different at $\alpha=0.05$. Analyzed by ANOVA with Duncan's new multiple range test (DNMRT) for multiple comparison of means.

positively with higher number of positive SPT reactions to these spore allergens (Table 5). The size of the SPT responses was also generally smaller among child than adult patients (data not shown).

Specific IgE quantification via FAST

To verify these SPT responses, we measured specific IgE to the spore and pollen allergens *in vitro* by FAST (Table 6). Spore allergen-specific IgE was detected in 40–90% of the individuals with the respective positive SPT responses to these allergens, and in none of the

healthy controls. The highest levels of allergen-specific IgE (FAST class 3 or >3 IU/ml and FAST class 4 or >17.5 IU/ml) were obtained for oil palm (*E. guineensis*) and common acacia (*Acacia auriculiformis*) pollen. The individual specific IgE levels and SPT responses (wheal and erythema sizes) were significantly correlated (Table 7).

Discussion

The aerobiologic survey of the Singapore environment showed the presence of a spectrum of fungi and fern spores and pollen that are unique to this region (16, 17). This is not surprising as Singapore is a tropical island city-state with climatic conditions that favor vegetation growth all year round and a characteristically diverse local flora. The use of commercial temperate pollen and fungal extracts for the evaluation of immediate hypersensitivity in our population has a very low frequency of positive response (19, 20). The need for a panel of allergens for the diagnosis of inhalant allergies in this tropical region is thus highlighted. This study has thus provided the basis for the delineation of the

Table 6. Specific IgE detection and levels

	Healthy controls† FAST + (%)	Asthma and/or allergic rhinitis patients†				Specific IgE (IU/ml)
		FAST + (%)‡	Class 1§	Class 2§	Class 3/4§	
Single species spore types						
<i>Cladosporium</i>	0 (0.0)	12 (6.8)	10	2	—	0.52 (0.35–0.91)
<i>Didymosphaeria</i>	2 (8.3)	27 (15.3)	12	13	2	0.94 (0.41–6.20)
<i>Pithomyces</i>	0 (0.0)	19 (10.8)	14	5	—	0.63 (0.37–1.10)
<i>Tetraploa</i>	0 (0.0)	14 (8.0)	11	3	—	0.49 (0.36–1.02)
<i>Curvularia</i> spp. spores						
<i>C. brachyspora</i>	1 (4.2)	38 (21.6)*	18	12	8	1.52 (0.36–21.21)
<i>C. fallax</i>	1 (4.2)	37 (21.0)*	19	11	7	1.48 (0.35–19.62)
<i>C. inequalis</i>	1 (4.2)	39 (22.2)*	18	15	6	1.55 (0.39–23.45)
<i>C. lunata</i>	0 (0.0)	37 (21.0)*	19	10	8	1.62 (0.38–20.90)
<i>C. pallescens</i>	0 (0.0)	37 (21.0)*	20	9	8	1.39 (0.36–17.69)
<i>Drechslera</i> -like spores						
<i>Bipolaris</i>	1 (8.3)	38 (21.6)*	20	12	6	2.31 (0.41–16.34)
<i>Corynespora</i>	1 (4.2)	38 (21.6)*	17	13	8	2.79 (0.35–19.52)
<i>Exserohilum</i>	0 (0.0)	26 (14.8)*	13	13	—	0.87 (0.38–1.42)
Fern spores						
<i>Asplenium nidus</i>	0 (0.0)	30 (17.0)*	17	13	—	0.51 (0.36–1.07)
<i>Dicranopteris curranii</i>	2 (8.3)	44 (23.3)*	13	11	17	2.10 (0.36–6.95)
<i>Dicranopteris linearis</i>	2 (8.3)	44 (23.3)*	13	12	16	2.68 (0.39–8.05)
<i>Nephrolepis auriculata</i>	1 (4.2)	38 (21.6)*	10	9	18	1.53 (0.35–4.95)
<i>Stenochlaena palustris</i>	0 (0.0)	29 (16.5)*	22	7	—	0.49 (0.35–1.00)
<i>Pteridium aquilinum</i>	0 (0.0)	29 (16.5)*	19	10	—	0.56 (0.35–1.75)
Pollen						
<i>Acacia auriculiformis</i>	0 (0.0)	51 (29.0)***	14	17	20	2.78 (0.36–17.26)
<i>Casuarina equisetifolia</i>	0 (0.0)	33 (18.8)**	18	11	4	1.15 (0.35–5.75)
<i>Kyllingia polyphylla</i>	0 (0.0)	37 (21.0)**	18	10	9	1.87 (0.39–4.29)
<i>Elaeis guineensis</i>	1 (4.2)	70 (40.9)***	23	18	29	3.57 (0.41–25.81)
<i>Podocarpus polystachyus</i>	2 (8.3)	59 (33.5)**	29	24	6	0.68 (0.35–4.57)

†Serum samples of 176 patients (92 children and 84 adults) and 24 healthy controls were collected and screened for specific IgE. Mean specific IgE levels (and range) shown are for samples which had positive responses (>0.35 IU/ml).

‡Cross tabulation of positive counts was evaluated by Fisher's exact test comparing patient groups with healthy controls. * $P<0.05$; ** $P<0.01$; *** $P<0.001$.

§FAST negative <0.35 IU/ml; FAST class 1 = 0.35–0.75 IU/ml; FAST class 2 = 0.76–2.99 IU/ml; FAST class 3 = 3.00–17.5 IU/ml; FAST class 4 >17.5 IU/ml.

Table 7. Spearman's rank correlation coefficients between specific IgE levels and skin prick reaction responses

Ranking	Extract	% skin prick positive†	Spearman's rank correlation coefficients (<i>P</i> value)‡	
			With wheal size (mm)	With erythema size (mm)
1	<i>Elaeis guineensis</i>	39.8	0.37***	0.49***
2	<i>Dicranopteris linearis</i>	34.2	0.22**	0.28***
3	<i>Podocarpus polystachyus</i>	33.8	0.15*	0.28***
4	<i>Curvularia inequalis</i>	31.6	0.21**	0.39***
5	<i>Bipolaris</i>	30.7	0.26***	0.32***
6	<i>Exserohilum</i>	30.7	0.32**	0.44***
7	<i>Dicranopteris curranii</i>	30.7	0.20**	0.29***
8	<i>Nephrolepis auriculata</i>	29.4	0.21**	0.31***
9	<i>C. brachyspora</i>	27.7	0.25***	0.33***
10	<i>C. lunata</i>	27.7	0.25***	0.29***
11	<i>Acacia auriculiformis</i>	27.7	0.31***	0.39***
12	<i>Casuarina equisetifolia</i>	27.7	0.32***	0.41***
13	<i>Didymosphaeria</i>	27.3	0.21**	0.28***
14	<i>C. fallax</i>	26.0	0.31***	0.35***
15	<i>C. pallescens</i>	26.0	0.29***	0.37***
16	<i>Asplenium nidus</i>	25.5	0.20**	0.28***
17	<i>Kyllingia polyphylla</i>	25.1	0.29***	0.36***
18	<i>Stenochlaena palustris</i>	21.6	0.21**	0.25***
19	<i>Pteridium aquilinum</i>	20.3	0.25***	0.29***
20	<i>Pithomyces</i>	19.9	0.11	0.15*
21	<i>Corynespora</i>	17.7	0.14*	0.31***
22	<i>Cladosporium</i>	16.5	0.12	0.35***
23	<i>Tetraploa</i>	16.0	0.14*	0.21**

†% skin prick positive among patients with atopic diseases.

‡**P* < 0.05; ***P* < 0.01; ****P* < 0.001.

environmental triggers of allergy in Singapore and the region.

The choice of pollen and spore allergens evaluated in this study was based on the frequency and abundance of each individual type found in our aerobiology survey. Out of more than 100 species of airborne spores and pollen, 23 of the most frequent and abundant were tested. SPT was used as the method of choice in this study as its results have been shown to correlate closely with respiratory allergies (13). Nevertheless, *in vitro* detection and quantification of specific IgE were also carried out to verify these results.

The overall prevalences of skin reactivity among individuals with atopic diseases to at least one of these allergens were 82.7%, 64.9%, and 68.0%, for fungi spores, fern spores and pollen, respectively. The higher number of fungal extracts used (12 out of the 23 extracts) may explain the higher frequency of responses to fungal spore extracts. However, a similar frequency of fungal spore responses among patients with atopic diseases of different age groups was also observed. The presence of these fungal spores, particularly the *Curvularia* spp. and *Drechslera*-like spores, in the indoor environment may result in significant exposure from a young age. Our data on the indoor mycologic environment in Singapore showed that the fungal spores present outdoors were also found indoors (unpublished data). In other studies, sensitization to fungal spores has also been found to occur as early as the age of 4 years (21).

Although a high proportion of the patients with atopic diseases were sensitized to the fungal spores, most of the reactions were weak. Despite their greater abundance, the lower frequencies and weaker responses to mold spore extracts than to pollen have also been reported elsewhere (22). In Singapore, fungal spores were found in the atmosphere perennially, with maximum spore loads reaching concentrations far above 1000 spores per cubic meter of air per day. Elsewhere, others have reported concentrations of over 100 000 spores per cubic meter of air even under "normal" conditions (22). Nevertheless, most mold-sensitive individuals have frequently been reported to have perennial symptoms of rhinitis and conjunctivitis (22), as in the pattern of allergic symptoms in Singapore (2).

Very little is known of the allergenicity of tropical pollen allergens, although studies have suggested that they are allergenic (23). The finding of a high frequency of sensitization to oil-palm pollen is unique to this study, and is likely to establish recognition of a novel pollen allergen. It is of interest to note that the atmospheric concentration of oil-palm pollen is positively correlated with wind speed and direction (17). It is likely that this pollen was blown in by the monsoon from Malaysia, where large plantations of oil palm are found. Our preliminary work on the characterization of the allergenic components of the local airborne spores, such as the oil-palm pollen, revealed multiple allergenic components (24). Chakraborty et al. (23) also demon-

strated the allergenicity of another species of palm pollen in Calcutta, India.

Another pollen, *Acacia auriculiformis*, had a relatively high frequency of sensitization. Other species of *Acacia* around the world have also been found to be allergenic. *Acacia* species in the southwestern USA are predominantly ornamental plants, which shed sufficient airborne pollen to sensitize patients suffering from pollinosis (25). In Israel, *Acacia* is also considered strongly allergenic (26), and it may be an important occupational allergen in Mediterranean regions (27).

Fern spores represent about 7% of the total outdoor airborne spores in Singapore and are present year-round, appearing to be common in the atmosphere of Southeast Asia (28). Fern-spore allergy has rarely been documented, and ferns have not been regarded as an important source of allergens, as their allergens are not readily extracted in solution. However, as shown in this study, sensitization to fern spores does occur and may be related to their abundance in the tropics. In Thailand, Bunnag et al. (28) showed that more than 70% of the atopic patients tested responded to extracts of fern spores, and allergy was substantiated by nasal provocation tests. In Israel, sensitization to spore extracts of ornamental ferns has been documented (29).

The frequency of sensitization to spore and pollen extracts was significantly associated with the presence of atopic disease and serum total IgE levels of the

subjects studied. These findings support the importance of these inhalant allergens in this region. However, sensitization rates to these allergens among young children (3–5 years) with atopic conditions were not significantly different from those of healthy controls. Moreover, the degree of reactivity among these children was observed to be generally weaker than for adults with atopic diseases. In contrast to indoor allergens such as dust mites (or even fungi), the frequency of sensitization to pollen and fern spores increased from childhood to adulthood. These results suggest that the development of pollen or fern spore sensitivity may require longer periods of exposure.

Although dust mites are the predominant sensitizing allergens, this study has identified the pollen and spore allergens of the Singapore environment. The association of these allergens with atopy suggests a role in the pathogenesis of allergic diseases in the tropics. Nevertheless, we are aware that a positive SPT response or the presence of circulating specific IgE does not necessarily lead to or indicate atopic disease. However, our current data showed that we were able to evoke allergic symptoms and responses in sensitized patients by nasal provocation challenge with extracts of these pollen and spore allergens (unpublished). Further characterization of their allergenic components is necessary for a better understanding of these allergens, and to assist in the standardization of allergenic extracts for diagnostic and therapeutic purposes.

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