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A Study of Pollen Prevalence in Relation to Pollen Allergy in Malaysian Asthmatics

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SUMMARY In this paper we report results of skin prick tests (SPT) using pollen extracts on 200 patients with clinical symptoms of asthma, and results of a parallel study in which pollen was collected and classified over a period of 18 months. The patients were outpatients from the University Hospital in Kuala Lumpur, Malaysia, while the pollen grains were collected with a spore trap placed in the campus of the University of Malaya, approximately one kilometer from the University Hospital. Pollen extracts of 3 grasses (Bahia, Bermuda, rough pigweed) and 2 flowering trees, *Acacia* and *Melaleuca*, were used in the SPT. Of the 29.5% asthmatics with positive SPT reactions, 21.5% were to one or more of the grass pollens, 21.5% to *Acacia* and 7.5% to *Melaleuca* pollen. *Acacia* and Bermuda grass extracts were the most allergenic, which agreed with results of the pollen collection which showed grass and *Acacia* pollen grains to be the two most commonly found pollens.

It has been established that indoor allergens, particularly from house-dust mites, are the most common allergens causing sensitization in asthmatics.[1,2,3] Other than indoor allergens, in temperate countries, pollen is the major factor in the outdoor environment which triggers allergic reactions, and there is a correlation between the quantity of pollen in a given environment and the incidence of allergic asthma.[4] Epidemiological data from 3 Southeast Asian cities[5] showed that house dust mites and cockroaches are by far the most common allergens causing asthma and allergic diseases, but interestingly, pollen was found to be significantly associated with eczema in Kota Kinabalu in Malaysia. We wanted to find out if pollen is an allergen affecting asthmatic patients in Kuala Lumpur in Malaysia, and whether allergy to pollen can be correlated to pollen prevalence. We report here the results of skin prick tests (SPT) on 200 asthmatic patients and pollen prevalence data from an 18-month study using a Burkard 7-day Recording Volumetric Spore Trap, which is designed to sample airborne particles continuously for periods of up to seven days without attention.

MATERIALS AND METHODS

Pollen sampling: A Burkard suction-type spore trap was installed on the roof of the Institute of Postgraduate Studies and Research building of the University of Malaya. It was set at an air intake of 10 liters per minute. After 7 days, the drum was removed and the adhesive-coated Melinex tape (Dow Corning) was detached and mounted in 7 sections. The airborne pollen was stained with Calberla's solution, two rows per slide were microscopically counted, and the number of pollen grains per cubic

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meter of air (Y), calculated using the formula[6] Y=(w.l.y)/(d.v.L) where w = width of the tape (14 mm), l = length of the tape corresponding to one hour (2 mm), y = estimated number of pollen grains in one lengthwise traverse, d = width of the lengthwise traverse (0.64 mm), v = volume of air collected during one hour (0.6 m^3), L = length of the tape corresponding to one day and night (48 mm).

For the duration of the sampling period, the weather was noted daily.

The Calberla stain was made up of 5 ml glycerol, 10 ml ethanol, 15 ml distilled water, 2 drops of saturated aqueous basic fuchsin and 2 drops of melted glycerine jelly.

The patient pool comprised 200 adult asthmatics from the outpatient asthma clinic of the University Hospital, who had not taken antihistamine in the previous 7 days. Informed consent was obtained before the SPT.

Allergenic extracts (Meridian, Texas) were dissolved in 50% glycerol. Histamine (1 mg/ml) and phosphate buffered saline in 50% glycerol were used as the positive and negative controls. Six µl of the extracts were pipetted on the volar surface of the forearm of the patient, a sterile syringe needle was then used to prick percutaneously. The diameter of the wheal was read after 15 minutes. A diameter of half or more than the size of the histamine wheal was considered a positive reaction.[7]

RESULTS

The pollen calendar (Table 1) shows the following order of prevalence during January 1996 to June 1997: Gramineae (grasses) > *Acacia* spp. > *Podocarpus* spp. > *Melaleuca* spp. > *Pandanus* spp. The Gramineae pollen grains accounted for 64.9% of the grains during January 1996 to June 1997 while *Acacia* accounted for 16.1 % and the rest was less than 10% each. There was no distinct pollen season, only minor fluctuations in frequency were observed (Table 1).

SPT: Twenty nine point five percent (59 of 200) of the asthmatics tested positive to one or more of the pollens. Twenty one point five percent (43 of 200) were positive to grass pollen (Bermuda grass 20.5%, rough pigweed 8%, Bahia

Table 1. Pollen calendar (number of pollen grains per cubic metre of air)

Month	Gramineae (all genera)	*Acacia* spp.	*Podocarpus* spp.	*Melaleuca* spp.	*Pandanus* spp.
Jan' 96	652	197	322	89	30
Feb	946	112	11	114	11
Mar	504	63	29	137	20
Apr	648	33	66	87	16
May	332	98	120	65	27
Jun	400	164	7	52	25
Jul	454	174	5	60	42
Aug	500	162	4	11	67
Sept	739	156	6	13	112
Oct	217	79	4	5	87
Nov	196	72	4	11	57
Dec	209	37	6	16	60
Jan' 97	283	59	4	25	46
Feb	403	53	8	19	34
Mar	614	76	2	19	25
Apr	355	55	6	8	19
May	139	165	246	9	19
Jun	380	222	2	11	32
Total	7,971	1,977	852	751	729

Table 2. SPT response to pollen and grass mix

Plant family	Allergens extract used in SPT	+ SPT (%)
Gramineae	Bermuda pollen	20.5
	Rough pigweed pollen	8
	Bahia pollen	6.5
	Mixed grass leaves	7
Leguminosae	*Acacia* pollen	21.5
Myrtaceae	*Melaleuca* pollen	7.5

grass 6.5%,), 21.5% to *Acacia* and 7.5% to *Melaleuca* pollen (Table 2). On the other hand, 7% were positive to a mixed-extract of leaves of 7 grasses (Bermuda, Johnson, Kentucky Blue, Timothy, Redtop, Orchard and Sweet Vernal).

DISCUSSION

The University Hospital and the University of Malaya building where the pollen grains were sampled are 1 km apart, and the majority of the patients attending the University Hospital asthma clinic live within a 10 km radius of the hospital, leading us to assume that the patients are exposed to pollen similar to that sampled. The identification of pollen grains is dependent on the shape, size and the characteristics of surface apertures, and the absence of these distinguishing features makes pollen identification complicated. For example, a pollen grain from the genus *Acacia* can be positively identified by its polyad (multigrain) structure of about 50 μm; a *Melaleuca* pollen grain by its 3 well-spaced round pores; and a *Podocarpus* pollen grain by its lateral air bladders. Pollen grains from the grass family Gramineae, however, are invariably small (20 to 32 μm), with only a small round aperture, and a lack of distinctive features which precludes identification beyond the family Gramineae.

The seasonal prevalence of pollen in Kuala Lumpur has been reported,[8] but in that study pollen grains were sampled by using the rotorod, which could sample only short periods of time, while we have used a spore trap that continuously sampled the air at a fixed rate of air-flow.

Twenty one point five percent of the asthmatics reacted positively in SPT to Bermuda pollen compared with only 7% reacting positively to the mixed grass leaves extract (which included Bermuda grass leaves). This is not surprising since pollen is the component that contains most of the allergens in a plant.[9]

An unambiguous correlation between pollen prevalence and the number of allergic asthmatics was not observed (data not shown). We noted that although 64.9% of the pollen grains sampled were grass pollen, only 21.5% of patients were allergic to grass pollen, compared with the same percentage (21.5%) of patients being allergic to *Acacia*, which accounted for only 16.1% of the pollen grains sampled. This could be due to grass pollen being less allergenic compared to *Acacia* pollen and to the inadequacy of using non-local pollen extracts in the SPT, in spite of us having selected grass and tree species that are closely related to those found locally. We conclude that 30% of asthmatics are allergic to grass and *Acacia* pollen, but the percentage may be higher if extracts of local plants are used in SPT.

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REFERENCES

1. Gelber LE, Seltzer LH, Bouzoukis JK, Pollart SM, Chapman MD, Platts-Mills TAE. Sensitization and exposure to indoor allergens as risk factors for asthma among patients presenting to hospital. Am Rev Respir Dis 1993; 147: 573-8.
2. Teoh PC, Tan WC, Oon CJ, Lui KF. Prick skin tests in bronchial asthma and their correlation with the specific serum IgE levels. Sing Med J 1977; 18: 228-31.
3. Leung R, Ho P, Lam CWK, Lai CKW. Sensitization to inhaled allergens as a risk factor for asthma and allergic diseases in Chinese population. J Allergy Clin Immunol 1997; 99: 594-9.
4. Ong EK, Knox RB, Singh MB. Molecular characterization and environmental monitoring of grass pollen allergens. In: Mohapatra SS, Knox RB, eds, Pollen Biotechnology gene expression and allergen characterization. New York : Chapman and Hall, 1996; 176-210.

5. Leung R , Ho P. Asthma, allergy and atopy in three South-east Asian populations. Thorax 1994; 49: 1205-10.
6. Ong EK. Grass pollen allergens: Molecular characterization and environmental monitoring. Ph.D. thesis, University of Melbourne, 1994.
7. Backman A. Skin tests for epidemiologic studies. Allergy 1994, 49: 493-94.
8. Ho TM, Tan BH, Ismail S, Bujang MK. Seasonal prevalence of air-borne pollen and spores in Kuala Lumpur, Malaysia. Asian Pac J Allergy Immunol 1995; 13: 17-22.
9. Marsh DG. Allergens and the genetics of allergy. In: Sela M, ed, The Antigens. New York: Academic Press, 1975; 3: 271-350.