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R. Prakashkumar ^a, P. M. Mathew ^b & P. Ravindran ^b

^a Tropical Botanic Garden and Research Institute, Palode, Kerala, 695 562

^b Department of Respiratory Medicine, Medical College & Department of Botany, University of Kerala, Thiruvananthapuram, India

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Studies on the allergenicity of nine tropical pollen allergens

R. PRAKASHKUMAR, P. M. MATHEW and P. RAVINDRAN

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Allergy evaluation studies using the antigens of nine commonly occurring airborne pollen grains in the state of Kerala, India were made. This includes the pollen grains of *Areca catechu*, *Casuarina equisetifolia*, *Elaeis guinensis*, *Heteropogon contortus*, *Oreodoxa regia*, *Peltophorum ferrugineum*, *Phoenix dactylifera*, *Spathodea campanulata* and *Swietenia mahagony*. Five hundred patients having respiratory complaints were selected for the study using these tropical pollen grains. Results were analysed in terms of age, sex, clinical history, family history and locality of the patients. The *Oreodoxa* antigen registered the maximum significant positivity followed by *Heteropogon*, *Casuarina* and *Peltophorum*. Age and family history of the patient had no direct influence in the positivity, but nature of the allergy, sex, phenological period and locality of the patient had a direct influence in hypersensitivity reactions. Similarly a very high positive correlation between Skin Test and Bronchial Provocation Test to these allergens among the tropical population was also proved. Thus the results established the role of these tropical allergens in eliciting Ig E mediated allergic reactions.

R Prakashkumar (Present affiliation: Tropical Botanic Garden and Research Institute, Palode 695 562, Kerala), P M Mathew and P Ravindran, Department of Respiratory Medicine, Medical College & Department of Botany, University of Kerala, Thiruvananthapuram, India.

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The role of pollen grains in eliciting nasobronchial diseases has been established. Of the innumerable species of plants whose pollen grains cause allergic conditions, only those which are wind pollinated are clinically significant. In order to cause the disease, such plants should be abundant in the environment of the susceptible persons so that the persons inhale their pollen while breathing. Hence a sound knowledge of the aerospora of a given region (aerobiology) and also an authentic information about the nature of the allergenicity of different groups of people would be most valuable for the effective clinical handling of allergic patients. The aerobiological survey of Kerala carried out by our team has yielded data on the common pollen and spore types (Prakashkumar et al. 1989, 1994, 1996; Ravindran et al. 1991). The present work, based on the results of the previous study, concerns the antigenic extraction and allergy evaluation studies on the newly identified pollen antigens among the patients of Kerala. The results obtained established the increasing intensity of the dermal and bronchial reactivity of the newly identified aeroallergens among the patients here. Using these nine different pollen antigens which were proved to be allergenic, diagnostic and therapeutic extractions now are being prepared here. These are now widely used among the respiratory allergic patients of Kerala for their ailments.

MATERIAL AND METHODS

The most abundant airborne pollen grains of *Areca catechu*, *Casuarina equisetifolia*, *Elaeis guinensis*, *Heteropogon contortus*, *Oreodoxa regia*, *Peltophorum ferrugineum*, *Phoenix dactylifera*, *Spathodea campanulata* and *Swietenia mahagony* were used in this investigation. A total of 500 patients underwent skin test for these

antigens. 290 of these were males and the remaining were females; i.e. a sex ratio of 1.38:1. The patients belonged to the age group of 10–49 years, and a majority of them were in the 20–39 age group (65.4%). 126 males (25.2%) and 102 females (20.4%) had a positive family history of allergic diseases. Communitywise classification showed 74.4% Hindus, 13.4% Christians and the remaining Muslims. The major patient group (N=291; 58.2%) suffered from Rhinitis cum Asthma, followed by 27.6% patients with Rhinitis and 14.2% with Asthmatic complaints.

Antigen extraction

The preliminary steps of antigen extraction include purification, drying and defatting of pollen grains. The extraction was carried out for 24–72 hours using PBS of pH8. These extracts were clarified by passing through millipore filters (pore size 0.22 mm) attached to sartorius filter adapters (Sartorius, FRG). Irritating and coloring materials which could stain the skin of the patient were removed by dialysis for 24 hours. Later the toxicity and aerobic and anaerobic sterility was examined using Soyabean Casein digest and Brewer Thioglycolate medium (Difco lab, USA). These extracts were lyophilised and later reconstituted as 1:500 for intradermal skin tests (Sheldon et al. 1967).

Protein estimation

2.5 ml of Alkaline Copper reagent solution was added to 0.2 ml of the protein sample. After 10 minutes incubation at room temperature, 0.5 ml of Folin – Ciocalteu reagent was added. After 30 minutes at room temperature the absorbance at 670 nm was measured. Finally the concentration of protein in mg/g dry pollen was calculated (Lowry et al. 1951).

Intradermal skin test

Intradermal skin test was performed on the volar aspect of the fore arm by injecting 0.02 ml of the antigen (1:500) using a sterile 1.0 ml tuberculine syringe. A negative control with PBS and a positive control with histamine phosphate (100 Mg/ml) was also used. The skin reactions were read after 15–20 minutes (Chai et al. 1975). The size of the wheal measured with a reaction gauge was graded by the following criteria.

Negative control	+	1+	2+	3+	4+
2	3–4	5–7	8–10	11–14	Above 14
3	4–5	6–8	9–11	12–15	Above 15
4	5–6	7–9	10–12	13–16	Above 16
5	6–7	8–10	11–13	14–17	Above 17

Reactions of 2+ and above are considered as significantly positive.

Bronchial Provocation Test

Inhalation challenge testing (BPT) is undertaken following significant positive skin testings. This is done by inhalation of an aerosol containing the antigen (which produced significant positive skin test reactions) which induces immediate hypersensitivity reactions in the lung leading to bronchospasm. Before bronchial challenge testing, the patient was asked to withhold bronchodilators at least for 8–12 hours and antihistamines and corticosteroides for 48 hours. Similarly the forced expiratory lung volume (FEV) of each patient is measured before the test. Only those having baseline FEV of 80% or more of the predicted value were considered for Bronchial Provocation Studies. This is first measured following the administration with five deep inhalations of the control aerosol consisting of the buffer saline diluent. This aerosol was generated by passing oxygen using an electronically triggered dosimeter, through the nebulizer containing the solution. If the patient showed a baseline FEV of 80% or more, then five deep inhalations of the selected antigens (1:500,000) were given and the lung function assessed at different intervals (5 min, 15 min, 30 min). The concentrations were serially increased (1:500,00, 1:5000) until a 20% fall in FEV1 occurred. A log dose response curve was plotted and the provocation dose producing a 20% fall in FEV1 was calculated by logarithmic graphic interpolation.

RESULTS

Incidence of skin test reactions in the present study showed an intermediate frequency of 38.35%. The *Oreodoxa* pollen antigen registered the highest frequency of 1+ skin test reaction (42.4%) followed by *Heteropogon* (39%), *Swietenia* (38.8%), *Spathodea* (34.2%) and *Casuarina* (33.6%). For the 2+ reactions also, *Oreodoxa* antigen showed the highest response (7.0%) followed by *Casuarina* (4.8%), *Heteropogon* (4.6%) and *Peltophorum* (4.4%). The *Phoenix* antigen registered the highest frequency of 3+ reactions (1.8%) closely followed by *Elaeis* (1.6%), *Swietenia* (1.4%) and *Casuarina* (1.2%). The *Elaeis* and *Peltophorum* types registered 0.4% 4+ reactions while *Phoenix* had only 0.2% 4+ reactions. As regards total positive reactions, the *Oreodoxa* antigen registered 50.2% followed by *Heteropogon* (44.2%) and *Swietenia* (43.8%) while the least was for *Areca* (25.4%). Considering the significantly positive reactions *Oreodoxa* also showed the highest percentage (7.8%) followed by *Casuarina* (6.0%), *Elaeis* and *Peltophorum* (5.4%) and *Heteropogon* (5.2%) respectively (Table I). The highest protein content was found in *Spathodea* antigen extracts followed by *Heteropogon*, *Peltophorum* and *Elaeis* respectively (Table II).

Among the 500 allergic patients studied here, a major group (N=394) with respiratory complaints came from the coastal belt of Kerala. The aerobiological studies of Kerala recorded major distribution of the pollen grains of *Casuarina*, *Oreodoxa*, *Phoenix* and *Swietenia* in the coastal belt of the state. The present study also reported maximum skin test positivities to these antigens among the patients from the coastal belt. The pollen grains of *Heteropogon contortus* was allergenic to the patients irrespective of the regional difference as these pollen grains were aerially recorded from all the regions of the state during the aerobiological study.

Significant correlation was observed between the skin test reactivity and flowering season of plants. Among those patients having clinical complaints during the flowering periods of *Casuarina*, 76.35% showed significant skin test positivities. Similar correlation was found for the antigens of the pollen grains of *Heteropogon* (56.25%), *Peltophorum* (67.51%) and *Swietenia* (84.73%).

Among the 2+ skin test reactions to each antigens the BPT positivity ranged from 72% to 83%. Similarly for 3+, the BPT positivity was from 85.71% to 100%, while for 4+ groups it was 100%. Considering all the 2+ skin reactions (N=169), almost 80% showed BPT positivity. For the 3+ reactivity (N=42) it was almost 100% and for 4+ reactivity there was 100% BPT positivity (Table III).

DISCUSSION AND CONCLUSIONS

The data on the frequency of skin test reactions in Kerala reported in this study show an intermediate frequency (38.35%) comparable to the figures of Hagy & Settupane (1969) and Mayers et al. (1986) for other countries. The data on different grades of skin sensitivity in Indian population show that 1+ reactivity for pollen antigens reported range from 0.44 to 33.55% (Anand et al. 1981; Present study), 2+ from 2.75 to 15.30% (Kulpati et al. 1979, Kundu et al. 1988) and 3+ from 0.30 to 6.30% (Shivpuri & Singh 1971, Kundu et al. 1988) and 4+ from 0.02 to 1.77% (Shivpuri & Singh 1971, Anand et al. 1981). There is a wider range in the intensity of the incidence of 1+ reactions in most cases. Higher number of 1+ reactions were previously reported among tropical population (Kundu et al. 1988, Shivpuri & Singh 1971). These 1+ dermal reactivities may not be due to hypersensitivity conditions but due to the presence of nonallergic factors in the antigen and the non specific reactions of the skin. Hence only significantly positive reactions were considered and the allergenicity confirmation of them was done through the Bronchial Provocation studies. Consequently the 1+ reactive patients were excluded from BPT studies. The magnitude of significantly positive reactions noticed here appears to compare rather well with other works in India (Kulpati et al. 1979, Shivpuri & Singh 1979 and Mittal et al. 1978). In the non allergic control group, the total positivity reports known so far (including present results) are by and large very negligible. In the present study, although the control group showed 1+ positivities (N=15; 5.50%), none of them showed any of the higher grades of reactivity. It may also be noted that none of these 1+ reactive controls did show any positivity with BPT. The magnitude of skin test reactivity among the allergic group

Table I. Summary data of skin test and BPT positivity with antigens.

No.	Name of Antigens	No. of tests	1+		2+		3+		4+		1+ to 4+		2+ to 4+		BPT positivity	
			No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
1	<i>Areca catechu</i>	500	123	24.60	4	0.8	—	—	—	—	127	25.4	4	0.80	3	75.00
2	<i>Casuarina equisetifolia</i>	500	168	33.60	24	4.8	6	1.2	—	—	198	39.6	30	6.00	26	86.66
3	<i>Elaeis guinensis</i>	500	166	33.20	17	3.4	8	1.6	2	0.4	193	38.6	27	5.40	23	85.18
4	<i>Heteropogon contortus</i>	500	195	39.20	23	4.6	3	0.6	—	—	221	44.2	26	5.20	20	76.92
5	<i>Oreodoxa regia</i>	500	212	42.40	35	7.0	4	0.8	—	—	251	50.2	39	7.80	33	84.61
6	<i>Peltophorum ferrugineum</i>	500	150	30.00	22	4.4	3	0.6	2	0.4	177	35.4	27	5.40	23	85.47
7	<i>Phoenix dactylifera</i>	500	131	26.20	9	1.8	9	1.8	1	0.2	150	30.0	19	3.80	17	89.47
8	<i>Spathodea campanulata</i>	500	171	34.20	17	3.4	2	0.4	—	—	190	38.0	19	3.80	15	78.94
9	<i>Swietenia mahagony</i>	500	194	38.80	18	3.6	7	1.4	—	—	219	43.8	25	5.00	19	76.00

Table II. Protein estimation.

Taxa	mg protein/g pollen
<i>Areca catechu</i>	08.20
<i>Casuarina equisetifolia</i>	07.18
<i>Elaeis guinensis</i>	10.85
<i>Heteropogon contortus</i>	11.70
<i>Oreodoxa regia</i>	05.06
<i>Peltophorum ferrugineum</i>	11.30
<i>Phoenix dactylifera</i>	09.11
<i>Spathodea campanulata</i>	14.36
<i>Swietenia mahagony</i>	07.63

Table III. Frequency of BPT response in patients having significant skin test positivities.

Taxa	Significant skin test positivities		
	2+ %	3+ %	4+ %
<i>Areca catechu</i>	75.00	—	—
<i>Casuarina equisetifolia</i>	83.33	100.00	—
<i>Elaeis guinensis</i>	76.47	100.00	100.00
<i>Heteropogon contortus</i>	73.91	100.00	—
<i>Oreodoxa regia</i>	82.85	100.00	—
<i>Peltophorum ferrugineum</i>	81.81	100.00	100.00
<i>Phoenix dactylifera</i>	77.77	100.00	100.00
<i>Spathodea campanulata</i>	76.47	100.00	—
<i>Swietenia mahagony</i>	72.22	85.71	—

was 3.7 times greater than that of the control groups (Hagy & Settupane 1969). But in this study the difference in reactivity observed was around seven times between these two groups.

Though the reactivity shown by the seven antigens were almost equal among the male and female patient groups, there was a slight increase in positivity among the male group. But for *Casuarina* and *Heteropogon* sp the positivity was significantly higher among the male patients. This could be due to the fact that males are relatively more exposed to outdoor air than females, who generally go outdoors less frequently. With respect to the clinical symptoms and reactivity, patients having rhinitis showed more positivity with *Areca*, *Elaeis* and *Spathodea* sp. Antigens of *Casuarina*, *Oreodoxa*, *Peltophorum* and *Swietenia* sp were more reactive among the asthmatic group while those with rhinitis cum

asthma and rhinitis alone reacted more with *Heteropogon* and *Phoenix* sp. No direct correlation between age and reactivity was observed. Workers like Kuhr et al. (1990) and Hanneuse et al. (1978) reported similar results. Although some patients with positive family history to allergic complaints reacted more, the difference in reactivity with the other group was not significant. Thus it appears that the pollen type rather than the family history influences the allergic response.

Convincing association between locality of the patients and their allergic response was evident with all the pollen antigens. The allergic patients studied here came from different localities and regions of Kerala, the majority were from the Coastal Belt (N=349). As regards the total skin test reactions, patients from the Coastal Belt registered the maximum frequency (40.49%) followed by those from Highlands (36.17%) and Midlands (32.30%). Concerning the reactivity with individual pollen types, it was noticed that the patients inhabiting the coastal belt region showed significantly positive response to the antigens of *Casuarina*, *Oreodoxa*, *Phoenix*, *Swietenia* and *Heteropogon* sp. Aerobiological studies done also reported the dominant occurrence of these pollen types in this coastal belt region. This indicated the positive correlation between aerospora of a particular locality and its influence on the allergic reactions among the people of the locality. A similar relation was also evident as regards flowering season of the plants. The patients who reported to the clinic with allergic complaints in the respective blooming periods were found to respond significantly to the respective pollen types. A similar correlation between flowering season of plants and their allergenicity has been reported for chestnut pollen by Klabuschnig et al. (1981) and also for other pollen types (Hill et al. 1979, Chapman & Williams 1984).

As significantly positive skin test reactions increased from 2+ to 4+ the BPT positivity also increased from 72% to 100%. For those patients with 2+ dermal reactivity to antigens, 72% to 83% BPT positivity was observed. For 3+ reactivity it was 85 to 100% and for the 4+ reactivity there was a 100% BPT positivity. As the significant skin test positivity increased from 2+ to 4+, there was an appreciable increase in the BPT positivity as well. This means that in diagnosing potent allergic reactions, it is superfluous to carry out both skin test and BPT. For routine diagnostic procedures, it would be enough to resort to the skin test method,

particularly in view of its easy performance with low cost and relatively less risk and complexity. A similar relation between skin test reaction and BPT has been pointed out by some previous workers, but their results were not as significant as the present findings (Aas 1970, 1975; Cavanaugh et al. 1977). Allergenicity of the various pollen types studied here has been evaluated in terms of the intensity of their skin test positivity followed by BPT studies. Our skin test data appear to show that the pollen of *Oreodoxa* sp. is the most potent allergen. The other types like *Casuarina* sp., *Elaeis* sp., *Peltophorum* sp., *Heteropogon* sp. and *Swietenia* sp. have more or less comparable allergenicity which could be rated next to *Oreodoxa* sp. The highly reactive *Oreodoxa* extract has a relatively low protein content while the least reactive *Spathodea* extract has the highest protein level. This may be due to that, the low amount of protein observed with *Oreodoxa* may contain more active allergenic constituents while the high amount protein observed with *Spathodea* may not have as much allergenic components. Thus this study revealed for the first time the intensity of allergenicity of these dominant airborne tropical pollen grains, the significance of aerobiological studies as a prerequisite in allergy evaluation studies and also the significant correlation between skin test and BPT among the tropical population. The plant to plant, person to person, and regional and seasonal variations in the intensity and incidence of different pollen found in this study indicate that such data may be considered valuable for effective clinical handling of allergy patients both in terms of advising preventive measures and for administering appropriate treatment schedules.

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