

THE JOURNAL OF
ALLERGY
AND
CLINICAL IMMUNOLOGY

VOLUME 95

NUMBER 2

Clinical aspects of allergic disease

***Anacardium occidentale* (cashew) pollen allergy in patients with allergic bronchial asthma**

Lalita Fernandes, MD, and Anthony Menezes Mesquita, MD
Panaji, Goa, India

Background: *The cashew tree grows in abundance in the hills and plains of Goa, India. Because of the financial yield, more and more trees are being planted each year. The cashew tree flowers once a year between January and March, but pollination is mostly entomophilous.*

Objective: *For the first time, a study was conducted to establish the possible role of the cashew pollen in triggering allergic asthma.*

Methods: *A stock solution of pollen extract was prepared with the standard weight/volume method for intradermal skin tests and a bronchial provocation tests (BPTs). The protein content of the antigen, estimated with the use of Folin phenol reagent and a spectrophotometer, was 28.72 mg/ml. Ten healthy volunteers and 65 subjects with allergic asthma, as documented by previous positive skin test reactions to various pollens, were studied.*

Results: *Of the 65 patients, 26 (40%) had positive skin test reactions in various grades. BPTs were performed in 22 of the 26 patients after their baseline peak expiratory flow volume was assessed. Twenty (90.9%) patients had a positive BPT result, and the majority of patients had grade III and grade IV reactions. None of the control subjects (n = 10) had positive responses to either intradermal tests or the BPT. Serum IgE levels, estimated by ELISA, were high in patients with positive skin test responses and showed a linear correlation with cutaneous sensitivity. Control subjects showed normal levels of IgE (39.0 ± 7.87 IU/ml).*

Conclusions: *This study provided us with knowledge of an additional pollen, *Anacardium occidentale*, which could trigger an asthmatic response in allergic individuals. (J ALLERGY CLIN IMMUNOL 1995;95:501-4.)*

Key words: *Cashew, allergic asthma, pollination, peak expiratory flow rate*

From the Department of T. B. and Chest Diseases, Goa Medical College, Panaji, Goa, India.

Received for publication Mar. 3, 1993; revised Nov. 11, 1993; accepted for publication Nov. 22, 1993.

Reprint requests: Anthony M. Mesquita, MD, Next to Indoor Stadium, Campal, Panaji, Goa, 403 001 India.

Copyright © 1995 by Mosby-Year Book, Inc.

0091-6749/95 \$3.00 + 0 1/1/55436

Demonstration that a pollen is a cause of allergic symptoms has traditionally been governed by Thommen's postulates, which are mainly concerned with the habitat of the plant and the production of windborne pollen in large inhalable amounts.¹ This study pertains to the allergenicity of pollen of an abundantly growing tree, *Anacar-*

Abbreviations used

BPT:	Bronchial provocation test
FEV ₁ :	Forced expiratory volume in 1 second
PEFR:	Peak expiratory flow rate.

dium occidentale (cashew) (Fig. 1, A and B). The cashew tree was a native plant of the tropical belt of Eastern Brazil and naturalized itself to various ecosystems in other parts of the world. It is of tremendous industrial value and hence has been grown extensively in public orchards, as well as in private courtyards and gardens. The flowers are small and yellowish pink with one-celled ovaries. Flowering is seasonal from January to March. Pollination is mostly entomophilous and, to a lesser extent, anemophilous.

In view of its widespread existence, a study was conducted for the first time to establish the allergenicity of its pollen and the ability of the pollen (in extract form) to induce a bronchial asthmatic response to a bronchial provocation test (BPT).

METHODS

Cashew pollen identification and counting were done by a palynologist using Durham's apparatus (gravitational method), which was kept at a distance of 5 m from the trees. Subsequently, with the assistance of a pharmaceutical research chemist, the cashew pollen antigen was prepared with the standard weight/volume method, which included grinding, defatting, extraction in buffered saline solution, filtration/clarification, and sterility testing. The protein content of the 1:50 concentration antigen was 28.72 mg/ml as determined by the Lowry method² with the use of Folin phenol reagent and a spectrophotometer.

Clinical aspects

Sixty-five patients with allergic bronchial asthma who earlier showed varying degrees of positive skin test reactivity to various allergens, ranging in age from 10 to 65 years (Table I), and 10 healthy volunteers who acted as the control group were studied after their informed written consent was obtained. All the routine precautions were taken before skin testing was performed. Antigen (0.02 ml of 1:500 concentration) and a control buffered saline solution were injected intradermally, and readings were taken after 20 minutes according to the criteria of Shivpuri³ (Table II) in both patients and control subjects. Patients who had positive skin test responses and a baseline peak expiratory flow rate (PEFR) of 80% underwent BPT after precautions were taken. Patients first inhaled buffered saline solution through a Pulmoaide nebulizer (De Vilbiss Medizinische GmbH, Langen, Germany) for 2 minutes, and PEFR was recorded. Because there is a good correlation between forced expiratory volume in 1 second (FEV₁) and



FIG. 1. A, Cashew tree (*A. occidentale*) with flowers. B, Close-up of flowers.

PEFR in assessment of airway response, we chose to measure PEFR (FEV₁ measurement could not be carried out at the time of intake because the machine was out of order).^{4,6} After 5 minutes, patients underwent antigen challenge with the graded dose technique (concentrations of 1:20,000, 1:10,000, 1:5000, 1:2550, 1:1000, and 1:500) until a 20% drop in baseline PEFR occurred or the patient showed signs of respiratory discomfort. After each BPT, patients were observed for any late-phase reactions for a period of 8 hours. These procedures were also carried out in the control group.

Serum IgE levels were estimated with the ELISA kit in both patients and control subjects.

TABLE I. Demographics of patients in the study ($n = 65$)

	Male	Female	Total
No. of patients	22	43	65
Place or origin			
Rural	21	40	61
Urban	1	3	4
Age (yr)			
Mean	30.09	21.53	25.81
Range	18-65	10-40	10-65
Duration of symptoms (yr)			
Mean	5.8	6.88	6.43
Range	3-12	4-14	3-14
Type of onset			
Perennial	26	35	61
Seasonal	3	1	4

TABLE II. Shivpuri's criteria for positivity

Grade	Criteria
0	Nearly same as control
1+	W: Twice the size of control E: ≤ 15 mm
2+	W: 3 times control E: > 15 mm
3+	W: 3.5 to 4 times control E: ≤ 30 mm
4+	W: 4 to 6 times control E: > 30 mm*

W, Wheal; E, erythema.

*Or any size with pseudopods.

RESULTS

The palynologic portion of the study showed a total cashew pollen count of 108 from January through March. Other pollens counted during the same period were *Graminae* (446), *Amaranthaceae* (112), *Compositae* (54), *Urticaceae* (46), *Palmae* (93), *Mimosae* (2), and *Caesalpineaceae* (140).

Of the 65 asthmatic patients evaluated, 61 (93.83%) complained of perennial symptoms, and four (6.17%) noted a seasonal history of asthma symptoms. Of these 65 patients, 26 (40%) had a positive response to *Anacardium* antigen as determined by intradermal skin testing. Of the 26 patients with positive skin test results, 24 (92.3%) lived in rural areas and only two (7.69%) lived in urban areas; 23 (88.46%) of them ($n = 26$) had perennial onset of symptoms, and only four (15.38%) had a seasonal onset (Table III). Detailed history did not reveal any mixed type of symptoms. Eight of the 26 patients were involved in harvesting or processing the fruits of the cashew tree. After careful history was

TABLE III. Demographics of 26 patients sensitive to *A. occidentale* antigen

	Male	Female	Total
No. of patients	10	16	26
Place of origin			
Rural	8	16	24
Urban	1	1	2
Age (yr)			
Mean	27.50	22	24.75
Range	16-42	12-32	12-42
Duration of symptoms (yr)			
Mean	6.2	4.88	5.54
Range	3-12	2-9	2-11
Type of onset			
Perennial	9	14	23
Seasonal	2	1	3
Involvement in harvesting, etc. ($n = 8$)	1	7	8

TABLE IV. Correlation of intradermal test grades to BPT

Intradermal test grades	No. of patients	BPT positive	
		No.	%
1+	3	2	66.66
2+	4	3	75
3+	8	8	100
4+	7	7	100
Total	22	20	90.9
Control subjects	10	0	0

Four patients refused consent for BPT.

taken, no specific correlation could be derived as to whether the attacks were worse during the flowering season because most of the subjects had a nonseasonal perennial type of asthma and also showed multiple pollen allergy reactions. Of the 26 patients with positive skin test responses, 22 underwent BPTs; four patients refused to give consent. Twenty patients showed a positive response to the BPT in various grades of skin sensitivity (Table IV). Two patients, one with grade I sensitivity and one with grade II sensitivity, did not respond to the BPT, even with allergen concentrations as high as 1:500. We did not observe any late-phase reactions after the challenge. None of the subjects in the control group had a positive skin test reaction or a positive BPT response. The serum IgE levels of the patients showed a linear correlation with the grade of cutaneous sensitivity.

DISCUSSION

In addition to the cashew pollen count of 108, pollen counts of *Graminae*, *Cassia* species, and

Amaranthaceae were 446, 140, and 112, respectively, during the same period. A high grass pollen count pertains to the fact that grass pollination is purely anemophilous and *Graminae* species are abundant throughout the hills and plains of this area. *Palmae* species, which are also abundantly grown and have an entomophilous type of pollination, showed a count of 93. *Compositae*, *Urticaceae*, and *Albizzia* species, though pollinated anemophilously, had comparatively lower counts during this period. This could be attributed to the lesser density of these plants.

The BPT results showed a good correlation with the grade of cutaneous sensitivity (i.e., 100% positivity in grade III and grade IV reactions). Similar observations were made by Niphadker et al.⁷ and Bryant et al.⁸ Two patients, one with grade I sensitivity and one with grade II sensitivity, failed to respond to the provocation test (Table IV).

In a similar study by Garcia-Ortega et al.⁹ in which the pollen antigen of *Mercurialis annua* was used, skin sensitivity to the pollen extract was detected in 8.5% of the 152 patients, and the BPT response was positive in 84.6%.

Data reveal that the relationship between airway responsiveness and skin sensitivity to allergen is not the same at all levels of skin sensitivity, even in subjects with comparable methacholine responsiveness. Hence, subjects with positive skin test responses frequently fail to react to BPT with the same allergen, whereas positive BPT responses with negative skin test results are unusual.¹⁰

On the whole, our study showed that the frequency of positive BPT responses in patients with positive skin test responses was 90.90%. None of the subjects in the control group had either a positive skin test or BPT response.

Another feature observed in atopic individuals is their ability to produce excessive amounts of IgE. Serum IgE levels measured in our patients, after presence of parasitic infestations and other diseases that could raise IgE levels was excluded, showed a linear correlation with skin sensitivity. Similar observations were made by Bapna et al.¹¹ and Brown et al.¹²

In summary, cashew pollen appears to be capable of inducing a clinical response as measured by bronchial challenge in patients who demonstrate positive skin test reactions to plant pollen. However, the significance of this finding requires further clinical correlation; but we certainly believe that cashew pollen could be a significant triggering factor of allergic asthma in patients working with

the plants during the pollination period or those living near the plantations.

This study provided us with knowledge of an additional pollen of *Anacardium occidentale* in the plant kingdom, which could trigger an asthmatic response in allergic individuals. However, the allergenic nature of this antigen could be further documented by direct RAST and RAST inhibition techniques. Immunoblotting could also be of value in characterizing the allergen in this IgE-mediated response.

We thank the Dean of Goa Medical College, Dr. V. J. Monteiro, for permitting us to carry out the research and publish the data. We also thank Mrs. Milan Saudagar, the palynologist, and Dr. B. P. Srinivasan, the pharmaceutical research chemist for helping in the identification and preparation of the antigen. We also thank the staff of the National Institute of Oceanography for their protein estimation of the antigen. This acknowledgement would be incomplete without our sincere thanks to the volunteers and patients for having consented to the tests.

REFERENCES

1. Norman PS. Discovering allergens. *J ALLERGY CLIN IMMUNOL* 1992;89:923-4.
2. Lowry OH, Rosenbrough NJ, Farr AL, Randall KJ. Protein measurement with Folin phenol reagent. *J Biol Chem* 1951;193:265-75.
3. Shivpuri DN. Comparative evaluation of sensitivity of common methods of diagnostic antigen tests in patients of respiratory allergy. *Ind J Chest Dis* 1962;4:102.
4. Speelburg B, Egidius AH, Panis MD, et al. Late asthmatic responses after exercise challenge are reproducible. *J ALLERGY CLIN IMMUNOL* 1991;87:1128-37.
5. Kelly LA, Gibson GJ. Relation between FEV₁ and peak expiratory flow in patients with chronic air flow obstruction. *Thorax* 1988;43:335-6.
6. Nowack RM, Penslar MI, Sarkar DD. Comparison of peak expiratory flow and FEV₁. *Ann Emerg Med* 1982; 11:64-9.
7. Niphadker PV, Narvekar AK. Role of BPT in diagnosis of nasobronchial allergy. *Lung India* 1985;3:21-4.
8. Bryant DJ, Burne MW, Lazarus L. The correlation between skin test, common allergens in patients with asthma. *Clin Allergy* 1975;5:145-7.
9. Garcia-Ortega P, Martinez A, Palacios R, Belmonte J, Richart C. *Mercurialis annua*: a new source of allergic sensitization and respiratory disease. *J ALLERGY CLIN IMMUNOL* 1992;89:987-93.
10. Fish JE. Bronchial challenge testing. In: Middleton E Jr, Reed C, Ellis EF, Adkinson NF Jr, Yunginger JW, eds. *Allergy: principles and practice*. 3rd ed. St. Louis: CV Mosby Company, 1988:1122-38.
11. Bapna A, Mathur US. The relationship of allergic bronchial asthma, cutaneous sensitivity and serum IgE. *Lung India* 1990;8:76-8.
12. Brown WG, Marilyn JH, Walter T, et al. The relationship of respiratory allergy skin test reactivity and serum IgE in a community population sample. *J ALLERGY CLIN IMMUNOL* 1979;63:328-35.