

Skin Reactivity to Antigenic Extracts of *Ricinus communis* Pollen Obtained from Diverse Source Materials

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Abstract

The allergenic potency of antigenic extracts derived from pollen of mature buds and flowers collected during the mid flowering season is higher as compared to that of antigenic extracts derived from the pollen samples either early or late in the season. There is similarity in the allergenic potency of the antigenic extracts derived from the pollen of mature buds and flowers collected during the same flowering season. The stored pollen showed markedly less allergenic potency as compared to the fresh lots. Similarly marked differences were observed in the allergenic potency among the antigens derived from the pollen samples from the plant populations growing in geographically distant localities of the country.

Key words : Pollen, Diverse sources, Quality control, Allergens, *Ricinus communis*

Pollen grains are amongst the earliest known allergens and are the major source of sensitization among atopic individuals^{1,2}. The various approaches suggested in control of pollen allergic diseases include allergen avoidance, medication to suppress symptoms and allergen specific immunotherapy³⁻⁶. However, the major lacunae in prevention and control of pollinosis is the poor quality control of antigens, which results in non-reproducible observations leading to incorrect diagnosis and failure in immunotherapy. This is because the preparation of antigenic extracts from pollen stored for many years are used. Moreover, the antigenic extracts prepared from one geographical location are used for skin testing and immunotherapy of patients in different geographical areas.

Ricinus communis (Euphorbiaceae), an important aeroallergen of India, was selected for the present study. It grows as weed along road sides and in vacant areas and is also cultivated in different parts of the country for its oil. The aim of the present study was to evolve quality control measure for manufacture of pollen antigen and to develop an effective and efficient allergen specific immunotherapy.

MATERIALS AND METHOD

Collection of Pollen

Pollen grains were collected separately from buds and flowers of *Ricinus communis* during the flowering season at 15 days interval. Pollen grains were also collected from different developmental stages of flower like immature buds (< 4mm in dia), mature buds (> 4mm in dia) and anthesised flowers separately on the same day.

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IJAAL, 2003, XVII (2) p 49-54.

The polleniferous materials were obtained from population of *Ricinus communis* growing at Bangalore, Bhopal, Nagpur, Tiruchirapally, Trivandrum and Vishakhapatnam. To assess the effect of storage on the quality of antigenic extract, pollen collected during 1984-1987 season and stored at 4°C after defatting were used.

Preparation of Antigenic Extracts

The pollen grains were first defatted with diethyl-ether till the solvent became colourless. The pollen suspension was filtered and pollen were dried in vacuum desiccator for 24 - 48 hrs.

Defatted pollen was suspended in 10% (w/v) phosphate buffer saline and continuously stirred at 4°C for 20 hrs on a magnetic stirrer. The suspension was centrifuged at 27,000 X g for 30 min at 4°C.

The supernatant was dialysed against distilled water in visking dialysis tubing (with cut off point 3500 daltons) for 24 hrs with frequent changes of distilled water. After dialysis the extracts were centrifuged again at 27,000 X g for 30 min at 4°C. The supernatant was then passed through millipore filter (0.22µ).

Intradermal Skin Tests

Patients with respiratory disorders attending Clinical Research Centre of V.P. Chest Institute (Delhi) were selected for skin tests. The patients were advised not to use antihistamines and tranquilizers (for 48 hrs) and other sympathomimetic drugs (8-12h) before the skin test performance. Patients of age group less than 15 years were excluded from the skin tests.

Intradermal skin tests were carried out on 110 patients with extracts of *Ricinus communis* pollen collected at different time intervals during the same season, different years and different populations in India. Reconstituted and sterilized antigens in 1:500 w/v concentration were used. The extracts were injected intradermally (0.01 ml each) on pre-cleaned volar surface of the forearm and anteriolateral part of the upper arm to make a bleb of 2-3mm. Phosphate buffer saline and histamine diphosphate (100µg/ml) were also injected as negative and positive controls,

respectively. Twenty healthy volunteers were also included in the testing to ensure that the antigens are devoid of non-specific irritants. The response of skin test was noted after 15-20 min of the test. The diameter of wheal and erythema produced were measured. The skin reactions were graded as per the criteria of Shivpuri⁷.

RESULTS

The range of variation in the patients showing markedly positive skin reactions (2+ and above) with the antigenic extracts of pollen derived from buds collected at different time intervals was 20%-38%, whereas 3+ and 4+ skin reactions were in 2.7%-15% of patients (Table 1). The variation observed in markedly positive skin reactions with the antigenic extracts of pollen derived from flowers collected at different time intervals was 20% - 37% and 4.5% - 9.0% in patients with 3+ and 4+ skin reactions (Table 2). The samples collected during mid flowering season (mid December - early February) showed higher values as compared to that of samples collected early and late in the flowering season. Except for one sample (25.10.87), all other samples did not show any positive reaction on normals.

The percent of patients showing markedly +ve (24%) and also 3+ and 4+ (3.6%) skin reactions were low for the antigenic extracts derived from pollen of

Table 1: Patients showing positive intradermal reactions (tested on 110 patients) to various antigenic extracts of pollen derived from buds of *Ricinus communis* collected during the same season

Date of Collection	Grades of skin reactions		
	1+ to 4+ %	2+ to 4+ %	3+ to 4+ %
02.10.87	41.0	27.0	05.0
25.10.87	40.0	26.0	04.5
26.11.87	50.0	25.0	04.5
24.12.87	50.0	29.0	08.0
23.01.88	48.0	38.0	15.0
09.02.88	45.0	03.0	09.0
20.03.88	38.0	20.0	02.7

Table 2: Patients showing positive intradermal reactions (performed on 110 cases) to various antigenic extracts of pollen derived from flowers of *Ricinus communis* collected during the same season

Date of Collection	Grades of skin reactions		
	1+ to 4+ %	2+ to 4+ %	3+ to 4+ %
02.10.87	41.0	20.0	4.5
25.10.87	43.0	27.0	5.4
26.11.87	43.0	26.0	3.6
24.12.87	45.0	36.0	7.2
23.01.88	43.0	37.0	7.2
09.02.88	45.0	33.0	9.0
20.03.88	36.0	22.0	5.4

Table 3: Patients showing positive intradermal reactions to antigenic extracts of pollen derived from different developmental stages of flowers of *Ricinus communis*

Developmental state	Number of patients tested	Grades of skin reactions		
		1+ to 4+ %	2+ to 4+ %	3+ to 4+ %
Immature buds	55	55	42.0	3.6
Mature buds	35	35	41.0	13.0
Anthesised flowers	37	37	46.0	8.1

immature buds as compared to that of the extracts derived from pollen of mature buds and flowers (Table 3). The antigenic extract from mature buds showed higher skin test values (13%) of 3+ and 4+ reactions as compared to that of flowers (8.1%), but the latter showed markedly positive reactions in 38% patients and the former produced these reactions in 32% of patients only.

The variation in percent of patients showing positive skin reactions to different antigenic extracts derived from pollen stored for varying period is given in Table 4. The patient showing markedly positive and 3+ and 4+ reactions varied from 15% to 38% and 0% - 8%, respectively. The samples collected during 1988, 1989 showed higher response as compared to stored pollen (1984 - 1987). None of the normals tested showed any positive skin reaction to extract tested. The range of variations in the patients showing markedly positive and 3+ and 4+ skin reactions to the antigenic extracts of pollen samples procured from geographically distant localities in India were 15%-39% and 3.6% - 17%, respectively (Table 5). The maximum response of markedly positive (39%) and 3+ and 4+ reactions (17%) was obtained from samples collected from Bhopal. The percent of patients showing markedly positive skin reactions to the antigenic extracts derived from pollen collected from Tiruchirapally was lowest (15%). None of the 15 normals tested showed positive response to any of the antigenic extract used.

Table 4: Patients showing different grades of skin reactivity to various antigenic extracts of *Ricinus communis* prepared from pollen stored for varying periods

Year of collection	Storage period (years)	Number of patients tested	Grades of skin reactions		
			1+ to 4+ %	2+ to 4+ %	3+ to 4+ %
1984	4	110	23.0	20.0	2.7
1985	3	110	38.0	16.0	2.0
1986	2	110	33.0	15.0	1.0
1987	1	110	32.0	15.0	0.0
1988	0 (fresh)	110	45.0	36.0	7.2
1989	0 (fresh)	37	46.0	38.0	8.0

Table 5: Patients showing positive intradermal reactions to antigenic extracts of *Ricinus communis* derived from pollen collected from geographically distant localities in India

Place of collection	Number patients tested	Grades of skin reactions		
		1+ to 4+ %	2+ to 4+ %	3+ to 4+ %
Bangalore	110.9	42.0	16.0	9.0
Bhopal	87	51.0	39.0	17.0
Delhi	110	45.0	36.0	7.2
Nagpur	84	33.0	20.0	5.0
Tiruchirapally	110	43.0	15.0	5.0
Trivandrum	54	35.0	24.0	6.0
Visakhapatnam	110	38.0	16.0	3.6

DISCUSSION

For standardization of pollen antigens it is important to assay the allergenic potency of the antigens and also to find out the magnitude of variation in the potency among antigens derived from the same species. Such studies lead to the quality control of pollen antigens and also pave the way for identification and characterization of allergenic extracts⁸⁻¹¹.

Although much attention has been paid to the final product of a pollen extract but no detailed study has been made on the quality of pollen source material. The various factors that affect the quality of pollen include pollen -authenticity, method and time of collection, processing and storage conditions¹². Keeping this in mind, intradermal skin tests were used to find out the variation in the allergenic activity of extracts derived from pollen of diverse sources.

The antigenic extracts prepared from the pollen of mature buds and flowers collected during mid flowering season of *Ricinus communis* showed markedly positive skin reactions in higher percent of patients as compared to that derived from pollen collected either too early or too late in the flowering season (Table 1). Similar observation for Antigen E (Ag E) content was made for *Ambrosia* anther extract by Larson¹³. They reported that Ag E, the allergenic component, is present in small amounts prior to pollination season, it increased with maturation of

anther and pollen grain and then declined gradually with the flowering season.

The fact that the antigenic extracts of pollen buds and anthesised flowers collected at different time intervals showed similar pattern of skin response suggest that bulk collection of pollen should include pollen from mature buds and flowers only. A similar observation was also made based on the similarity in protein content, IEF and SDS-PAGE protein profiles¹⁴.

As compared to earlier reports^{15,17} the percent of patients showing markedly positive skin reactions to pollen antigen of *Ricinus communis* is higher (20%-38%) in the present study. This again lay emphasis on the importance of quality raw material for antigen - preparation. Immature buds should not be included in the bulk collection of pollen as the biopotency of the antigen derived from their pollen is markedly less. However, there is a similarity in the biopotency of pollen antigens of mature buds and flowers.

The result on the response of patients tested with the antigenic extracts derived from pollen stored for varying periods showed that storage the potency of antigenic extract decreases. The loss could be due to leaching and degradation of allergenic components during storage. Anderson¹² reported that both total protein content and amount of allergenic protein (Ag E) varied significantly in short ragweed samples stored for 1959-1968. Similarly variable protein content in six extracts of *Ambrosia* pollen stored for different years was also observed¹⁸.

The pollen antigens derived from plants growing in geographically distant localities across latitudinal and ecological gradient also differ in the allergenic potency. The antigenic extract prepared from pollen procured from Bhopal and Delhi showed markedly positive skin reactions in higher number of patients (39% and 36%, respectively) whereas from other localities skin reaction was recorded in 15%-24% of cases. The geographical variation in allergenicity of I pollen antigen has also been reported by earlier workers. From Bhopal 19% of patients showed the skin reaction with pollen antigens of *Ricinus communis* whereas 12%, 20%, 24% and 42% of patients showed positive skin reactions to pollen antigen at Andhra Pradesh, Varanasi, Aligarh and

Lucknow, respectively¹⁹⁻²³. From the same locality (Kanpur), Gupta *et al*²⁴ reported that 13% of patients showed positive skin reaction where as Mittal *et al*²⁵ reported that only 6% of patients show positive skin reaction to pollen antigen of *Ricinus communis*. Similar variability in the allergenic potency of pollen antigen was reported from Lucknow^{20,26}.

In all the skin tests performed on healthy volunteers except for two individuals who showed 1+ reaction to antigenic extracts of *Ricinus communis*, all others showed negative response. Several workers have also reported 1+ skin reaction on normals with pollen antigens²⁷⁻²⁸. The dilution factor 1:500 w/v has been commonly employed in intradermal skin tests as high antigen concentration not only induces false positive reactions but also generate anaphylactic shock in hypersensitive patients^{28,29}. Our observations also reveal that the sensitivity with 1:500 w/v dilution is appropriate without any side effects on patients⁷. Consequently this dilution factor can be used in the intradermal skin tests.

ACKNOWLEDGEMENTS

Authors are thankful to Dr. D. Parkash, V.P. Chest Institute for his clinical help.

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