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POLLEN ALLERGY OF *CYNODON DACTYLON* (L.) PERS.

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Allergy, though a commonly occurring disease, the real cause for its occurrence in most parts of the world is found to be the multitude of biological particulate matters, especially, the pollen grains. In order to, disclose the real role of pollen of *Cynodon dactylon* in inciting the allergic symptoms in the susceptible allergic patients of Puducherry region, 105 patients and 45 normal controls, were subjected to Skin Prick Test (SPT) with the antigenic extract of pollen along with the positive and negative controls. Thirty three patients showed positive reaction to SPT by Wheal formation of 3x3 mm to 6x6 mm. No flare was observed in any of the patients and in the normal controls. SDS – PAGE profile of the pollen extract showed prominent protein bands of 66 and 43 KDa (Kilo Dalton). Dot blotting also showed prominent binding between the antigen and the antibody. The average total IgE (Immunoglobulin E) in patients was 752.3 IU/ml (International Unit per milli litre) and in normal controls it was 380.2 IU/ml. The SPT and immunobiochemical analysis confirmed that *Cynodon dactylon* is a source of pollen allergen.

Keywords: *Cynodon dactylon,* Dot Blotting; Pollen allergy; Protein separation; Skin Prick Test; Total IgE estimation.

Introduction

Even though, the inhabitants enjoy the pleasure of the natural vegetation, somehow they are unknowingly affected by the trivial things produced by the vegetation such as pollen grains which are carried by wind far and wide. These pollen grains, which have direct contact with people, may bring out allergic symptoms, which are associated with runny nose, watery itchy eyes, skin rash and bronchial asthma. To overcome the allergic diseases and to safeguard the life of susceptible allergic individuals, it has become mandatory to study the vegetation and the climatic factors of a particular place and then to estimate the richness and percentage of the pollen in the atmosphere. The only ideal way to avoid the allergic diseases is the avoidance of pollen grain but it is much difficult to achieve in the open pollen laden atmosphere. Hence, it is necessary to study the relation of pollen in causing allergy in humans.

A positive correlation between the clinical symptoms of the allergic patients and the concentration of airborne pollen was reported by several investigators[1-6]. In India allergenically potent pollen producing plants are fairly common. The allergenic potentiality of *Acacia auriculiformis*, *Eucalyptus citriodora* and *Madhuca indica*[7], *Amaranthus spinosus*[8], *Salmalia malabarica*[9] , *Sorghum vulgare*[10] , *Ricinus communis*[11] and *Mallotus phillipensis*[6] has been well established. Thus, to establish the extent of the effect of pollen allergens on local inhabitants of Puducherry region, a study on the pollen of *Cynodon dactylon* (L.) Pers. (Bermuda grass) of the family Poaceae was carried out for the very first time.

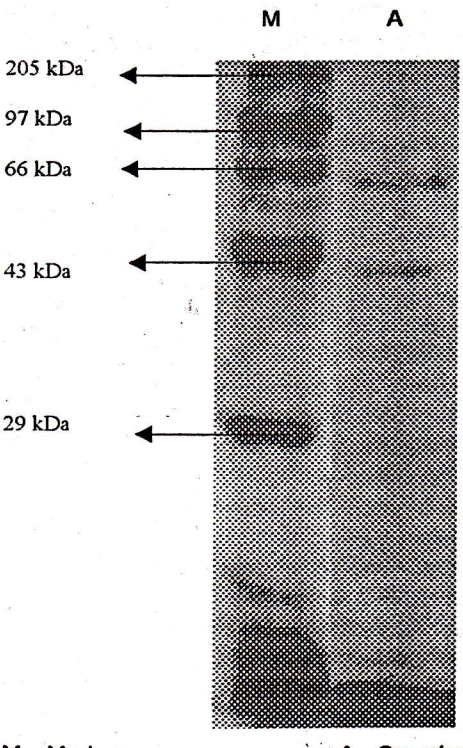
Materials and Methods

The study includes Skin Prick Test (SPT), pollen antigen extraction and estimation, dot blotting and total immunoglobulin E estimation (IgE). Pollen grains were collected from the inflorescences of *Cynodon dactylon* and were stored properly to prepare antigenic extract. 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 desiccators for 24-28 hrs. Defatted pollens were 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 Xg for 30 minutes at 4°C. The supernatant was dialyzed against distilled water in visking dialysis tubing (with cut off point 3500 Dalton) for 24 hrs with frequent changes of distilled water. After dialysis the extracts were centrifuged again at 27,000 Xg for 30 minutes at 4°C. The supernatant was then passed through Millipore

Table 1. Skin prick test with *Cynodon dactylon* pollen antigen.

Sl. No.	Age	Wheal Size (mm)												No Reaction	
		1 x 1		2 x 2		3 x 3		4 x 4		5 x 5		6 x 6			
		P	C	P	C	P	C	P	C	P	C	P	C	P	C
1	11-20	1	4	3	3	1	-	2	-	-	-	-	-	2	10
2	21-30	2	3	4	4	-	-	-	-	3	-	1	-	4	13
3	31-40	3	1	7	-	1	-	3	-	3	-	1	-	5	4
4	41-50	2	1	5	1	1	-	-	-	-	-	1	-	6	1
5	51-60	2	-	6	-	7	-	-	-	-	-	1	-	7	-
6	61-70	1	-	6	-	2	-	1	-	-	-	-	-	3	-
7	71-80	2	-	-	-	3	-	1	-	-	-	1	-	1	-
	Total	13	9	31	8	15	0	7	0	6	0	5	0	28	28

Note: P – Patients C – Controls



M – Marker A - *Cynodon dactylon*

Fig.1. Protein bands of *Cynodon dactylon* pollen antigen.

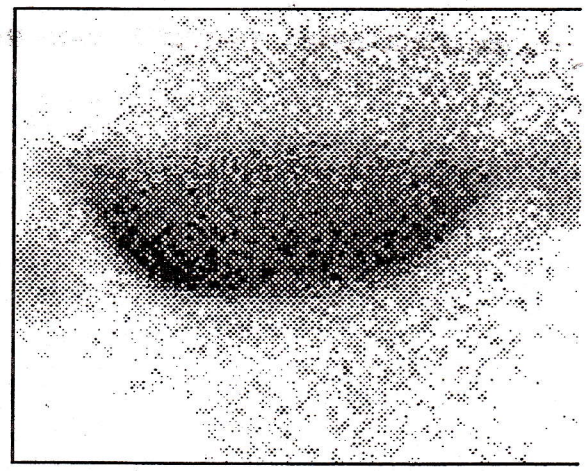


Fig.2. Binding of *Cynodon dactylon* pollen antigen with the antibody of serum of patients.

filter (0.22μ) and stored at 4°C for SPT [6].

SPT was carried out to find out the potentiality of the pollen antigenic extract in inducing allergic reaction (Wheal and Flare reaction) and indirectly to find out the extent of susceptibility of allergic reaction in Bronchial Asthma patients and in normal controls. The patients attending the Out Patient Department (OPD) of Jawaharlal Institute of Post Graduate Medical Educational and Research (JIPMER), Puducherry with the symptoms and

complaints suggestive of Allergic Asthma were subjected to SPT. A total of 105 Bronchial Asthma patients and 45 normal controls were tested with the antigenic extract, positive control (Histamine) and negative control (Phosphate Buffer Saline). Informed consent was obtained from the patients before enrolment in the study. A predetermined proforma containing details of allergies and clinical condition was filled up before SPT. SPT was performed using Quintip needle, on the cleaned (75% Ethyl Alcohol) volar surface of the forearm of females and on the back of the body of males. After 15 minutes, the dimension of Wheal and Flare were measured using Vernier Caliper. Five ml of blood was collected from each of the positive allergic patients and also from the normal controls for further studies. The sera were separated and stored at – 86 ° C.

The amount of protein in the antigenic extract was estimated by the method of Lowry *et al.*,[12] using Bovine Serum Albumin as standard. The antigenic extract was subjected to SDS – PAGE to screen out the protein bands. Briefly 12% SDS – PAGE was prepared and run on SDS - PAGE mini protein Electrophoresis Apparatus (Biorad – Diagnosis, USA). Protein bands were stained by Commassie stain and the bands were directly spotted on the Nitrocellulose membrane. The blotted membrane was hybridized with the blood serum of the patients showing positive SPT to the pollen extract to identify the presence or absence of the homologous fragments. Increased level of total IgE, which is a direct measure of extent of allergen and its role in causing allergy diseases, was studied through ELISA. Total IgE of well stored blood sera of patients and normal controls were estimated.

Results and Discussion

The preliminary clinical study of SPT showed significant positive results of allergic reaction. The wheal size ranged from 1x1 mm to 6x6 mm in the patients tested with antigenic extract (Table 1). A wheal size of 3x3 mm in 15 patients; 4x4 mm in 7 patients; 5x5 mm in 6 patients and 6x6 mm in 5 patients were observed. In 17 normal controls a maximum wheal size of 2x2 mm was observed. In order to know that SPT is not because of false positive reaction, the blood sera of the patients were studied for the IgE antibody by ELISA. An average total IgE of 752.3 IU/ml in patients and an average of 380.2 IU/ml in normal controls were observed. The protein profile of SDS-PAGE showed two bands of molecular weight of 66 KDa and 43 KDa (Fig. 1). The serum of patient reacted with the protein bands of pollen antigen and the binding was significant (Fig. 2).

According to Susan Varela[13], a wheal of at least 3 mm by 3 mm is a positive reaction. Of 105 patients observed 33 showed wheal dimensions of 3x3 mm to 6x6 mm. It is suspected that one or both of the protein bands might be involved in inciting allergic symptoms in the sensitive patients. Binding of serum of patients and protein bands of pollen extract is indicative of allergy. The increased level of total IgE further confirms that the pollen is allergic. According to Chowdry *et al.*,[14] healthy non allergic adults have an expected total IgE up to 120 IU/ml. The higher IgE level in normal controls in India is explained probably by the higher incidence of parasitic infestations. Thus, the SPT and immuno-biochemical analysis confirm that *Cynodon dactylon* is a source of pollen allergen.

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