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ARTICLE

Evaluation of hypersensitivity due to *Peltophorum pterocarpum* (DC.) K. Heyne pollen grains using intradermal skin testing.

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Abstract

Nasobronchial allergy and associated illness are considered to be the most ancient but relevant health diseases of human beings.Aerobiological studies conducted in Kerala (India), revealed the incidence of pollen grains of *Peltophorum pterocarpum* (DC) K. Heyne. belonging to the family Fabaceaeas a common airborne pollen member. Clinical trial conducted in different parts of the world also proved its allergic potentiality among human beings. Present study concerned with the allergic evaluation of this particular airborne pollen type among Keralites. Intradermal skin test results of 1500 patients having the history of respiratory complaints was collected for the allergy evaluation study. Results showed that this particular pollen type is highly potent allergen and the degree of reactivity is depends the locality in which the patients inhabits.Allergic response this pollen type was assessed in terms of various parameters such as sex, age, family history, clinical history and locality of the patients. It was observed that, significant skin test positivity is high among male patients. The overall skin test response in this study indicate that the allergic response is independent of the age of the patients. Patients with clinical history of asthma showed maximum reactivity. The skin test results indicate that family history of patients has no significant influence on the skin test positivity.

Keywords: Pollen allergy, Intradermal Skin testing, Positivity.

Introduction

An allergic reaction refers to an exaggerated reaction by our immune system in response to the contact with certain foreign substances. It is exaggerated because these foreign substances are usually seen by the body as harmless and no response occurs among non-allergic group. The concept of allergy was originally introduced during 1906-1907 by the Viennese pediatrician, Clemens Von Pirquet, after he noticed that some of his patients were hypersensitive to normally innocuous entities such as dust, pollen grains or certain food substances. He called this phenomenon "allergy" and the term was derived from the ancient Greek words "allos" meaning different or changed and "ergon" meaning work or action. Usually allergic response occurs when the body of such people recognize the foreign substances and one part of their immune system is turned on. Since then allergic reactions were considered to be an immunological abnormality.

Materials that are capable of trigger allergic reactions are referred as allergens and are most commonly airborne. Such types of airborne allergens are commonly called as "aeroallergens". Nature of aeroallergens varies from place to place depending upon the environmental, climatic and other geographical conditions. Majority of allergic manifestations may happen due to the inhalation of particles like reproductive spores of plants and fungi. In Canada and United States, the commonest cause of nasobronchial allergy is the Rag weed pollen. Among grasses, Timoty and June grasses are Studies conducted around the globe indicate that about 20% of world population suffers from one or other forms of allergic disorders. This include rhinitis, bronchial asthma, allergic alveolitis, rhino sinusitis, conjunctivitis, atopic dermatitis and food or drug allergies (Barkin and Mc Grovern, 1966; Haahleta, 1979; Singh et al, 1994). In India, statistics of the allergic conditions state that about 10% of the population has been estimated to suffer from allergic disorders (Viswanathan, 1964).

Kerala, Southernmost state of India, is characterized for its diversity in flora and fauna. Despite this diversity little study is conducted which involve the characterization of different pollen types present in the environment. Aerobiological studies conducted at the Department of Respiratory Medicine during late eighties and early nineties, revealed the presence of large number of pollen grains in the atmosphere. Clinical studies proved that, about 70-80 % of them are potent allergens (Ravindranet al., 1986, 1988; Nair et al, 1986, Gopiet al, 1992 and Prakashkumar, 1989, 1993). These became the foundation for the present study. Among them a large number of pollen samples belong to the family Poaceae, Fabaceaeand Arecaceae were identified.

The present work concerns the antigenic extraction and allergy evaluation by intradermal skin testing of the pollen

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grain types of *Peltophorum pterocarpum* (DC.)K. Heyne. Analysis of allergenic response among a sample of 1500 patients who reported at the allergy and Applied Immunology clinic of the Department of Respiratory Medicine, Medical College, Thiruvananthapuram. It is expected that the study bring forth results relevant to the signal areas of diagnosis and clinical management of allergic reactions due to this pollen type.

Materials and Methods

Materials

Of the innumerable species of plants whose pollen grains are allergenic, only those which are wind pollinated are clinically significant. In order to cause the disease, such plants should be abundant in the environment and also must produce large quantities of pollen grains. Considering the above facts, pollen grains of *Peltophorum pterocarpum* (DC.)K. Heyne. belonging to the family Fabaceae was selected for the present study.

Peltophorum pterocarpum (DC.) K. Heyne. commonly known as copperpod, yellow-flamboyant, yellow flame-tree, yellow poinciana or yellow-flame, is native to tropical south-east Asia and northern Australasia. The tree is widely grown in tropical regions as an ornamental tree, particularly in India, Nigeria, Pakistan, and Florida and Hawaii in the United States. Used as decorating flower in Telangana State's Batukamma festival.The trees have been planted alternately in India as a common scheme for avenue trees in India alternately with Delonix regia (Poinciana) to give a striking yellow and red effect in summer.This upright, handsome, spreading, semievergreen tree has a rounded canopy and is capable of reaching 50 feet in height with a 35 to 50-foot spread. The dark green, delicate, feathery leaflets provide a softening effect for the tree's large size and create a welcoming, dappled shade. From May through September, the entire tree's canopy is smothered with a yellow blanket of flowers, appearing in showy, terminal panicles and exuding a delicious, grape-like perfume. These flower clusters are followed by four-inch-long seed pods which ripen to a brilliant, dark, wine-red.

Fresh inflorescence *Peltophorum pterocarpum* (DC.)K. Heyne.were collected in triplets from different localities of the Highlands, Midlands and Coastal belt of Kerala State. From the flower, pollen grains were collected in bulk for further investigations.

Collection, Purification, drying and defatting of pollen grains

The pollen were collected in bulk, air dried and sieved to remove the debri. Collected pollen grains were passed through 100, 200 and 300 mesh sieves. Purity was confirmed by observing through a microscope, with 99% pure being considered as A-grade pollen. Proper dehydration was done by keeping the pollen grains in a hot air oven at 60°C for one to three hours, which prevents microbial contamination. Purified pollen grains were defatted in Petroleum ether, which was later filtered, evaporated and dried. These steps were repeated thrice until defatting of pollen grains is completed.

Antigen extraction.

Antigenic extraction of these pollen grains were made in Phosphate Buffered Saline of pH 8.0 following Sheldon et al. (1967). Known amount of pollen grain was put in PBS, mixed thoroughly and the extraction was done for 72 hours at 4°C. After extraction, the extract was filtered and clarified by passing through millipore filters of pore size 0.45mm attached to Sartorius filter adaptors (Sartorius, Germany.). For lyophylisation, the clarified products were dialyzed using dialysis tubes of 27×32" tubings, for 24 hours. Lyophylised products were constituted later as 1:500w/v for clinical. The extracts were sterilized using proper reagents. The aerobic and anaerobic sterility testing using Soyabean Casein Digest medium (Himedia Lab, India.) and Brewer Thioglycolate medium (Difo laboratories, Detroit, Michgan, USA) was done after filtering the extracts through millipore filters of 0.22mm.

Allergy Evaluation

a. Sample selection

A total of 1500 patients were selected from those attending the Department of Respiratory Medicine, Medical College, Thiruvananthapuram, Kerala, due to allergic complaints.

b. Inclusion - exclusion criteria

The patients were selected by definite inclusion - exclusion criteria. Patients of age between 10-49 years and a history of respiratory allergy were included. Patients of chronic asthma for 10 years or more, patients below 10 and above 49 years and patients on daily steroids were excluded. Also those having associated illness like food allergy, drug allergy and other associated problems were excluded from the study.

c. Intradermal skin test (ID)

Allergy evaluation was done based on the procedure of Chai et al. (1975). The patient is seated comfortably, cleaned with 70% alcohol and allowed to dry evaporation. The upper half of the volar surface of the fore arm was selected as the test sites and were marked leaving sufficient space. 1:500 dilution of antigenic extract was used for testing. Phosphate Buffered Saline (PBS) and histamine phosphate (100µg/ml) were used as negative and positive controls respectively.

Sterile 1ml glass tuberculine syringe with 26 gauge needle were filled with 0.1ml of the test solution. All the air bubbles were expelled fully to eliminate misleading splash reactions which could be interpreted mistakenly as abnormal. Stretched the skin and the syringe was placed an angle of 45° to the arm, introducing the needle into the skin. Advanced the needle until the entire level of the needle was into the skin penetrating entirely, but not going deeper than the superficial layers of the skin, since subcutaneous injec-

tions can lead to a false negative test. Injected gently a small amount of allergen solution that can raise a bleb of 1-3 mm diameter (approximately 0.02ml of solution). But if no wheal is formed immediately after injection, the needle was withdrawn and repeated the test at a different site. The skin reactions were read after 15 to 20 minutes. The degree of reactivity was calculated by measuring the wheel size after 20 minutes and interpreted based on Chai et al. (1975) (Table 01). The size of wheal was measured with a reaction gauge. The pseudopods, erythema (if occurs) were also noted.

Results and Discussion

Respiratory ailments due to the inhalation of various foreign substances is a serious concern among people across the globe. There are many forms of allergic manifestations among which allergic rhinitis and bronchial asthma are important.It has been well established that allergic problems are often caused by the inhalation of various substances which is grouped under two heads such as biotic and abiotic. Among the biotic factors, reproductive spores of various plant organisms hold a major role to play. Studies conducted during these years have shown that many pollen types are potentially allergic in various degrees (Cvitanovicet al, 1986; Tiwari, 1978; Agashe and Anand, 1982; Meyers et al, 1986). Intensity of hypersensitivity reactions can be determined by various diagnostic tools, among which intradermal skin testing is considered to be the most relevant and accepted procedure.

The incidence to the skin test response to *Peltophorum pterocarpum* (DC.)K. Heyne. antigen among the patients is presented in table 02. Among the 1500 patients subjected to intradermal skin test with the pollen antigen of *Peltophorum pterocarpum*, 696 patients (46.40%) reacted positively. Among this a group of 160 patients (10.67%) showed significant reactivity in the skin test (Table: 02).

When individual reactions were taken into consideration, highest positivity of 1+ reactivity was shown by 536

Table 1. Criteria to read Intradermal Test

Control (mm)	±	1+	2+	3+	4+& above
2	3 – 4	5 – 7	8 - 10	11 – 14	Above 14
3	4 – 5	6 – 8	9 – 11	12 – 45	Above 15
4	5 – 6	7 – 9	10 – 12	13 – 16	Above 16
5	6 – 7	8 – 10	11 – 13	14 – 17	Above 17
6	7 – 8	9 – 11	12 – 14	15 – 18	Above 18
7	8 – 9	10 – 12	13 – 15	16 – 19	Above 19

Table 1. Incidence of Intradermal Skin test reactions to *Peltophorum pterocarpum* (DC) K. Heyne pollen antigen

Reactivity	No.	%
-ve	804	53.60
1+	536	35.73
2+	127	8.47
3+	24	1.60
4+	09	0.60
Total Positivity	696	46.40
Total Significant Positivity	160	10.67

Table 3. Age and nature of reaction to P*eltophorum pterocarpum* (DC) K. Heyne

Age	1+		2+		3+		4+ and above		Total positivity		Total significant positivity	
	N	%	N	%	N	%	N	%	N	%	N	%
10 – 19 Yrs	111	7.4	29	1.93	05	0.33	-	-	145	9.67	34	2.27
20 – 29 Yrs	158	10.53	31	2.07	05	0.33	-	-	194	12.93	36	2.40
30 – 39 Yrs	130	8.67	37	2.47	08	0.53	05	0.33	180	12.00	50	3.33
40 – 49 Yrs	137	9.13	30	2.0	06	0.40	04	0.27	177	11.80	40	2.67
Total	536	35.73	127	8.47	24	1.60	09	0.60	696	46.40	160	10.67

Table 4. Sex and nature of reaction to P*eltophorum pterocarpum* (DC) K. Heyne

Age	1+		2+		3+		4+ and above		Total reactivity		Total significant reactivity	
	N	%	N	%	N	%	N	%	N	%	N	%
Male	308	20.53	81	5.40	15	1.00	07	0.47	411	27.4	103	6.87
Female	228	15.02	46	3.07	09	0.60	02	0.13	285	19.00	57	3.50
Total	536	35.73	127	8.47	24	1.60	09	0.60	696	46.40	160	10.67

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Table 5. Clinical history and reaction to *Peltophorum pterocarpum* (DC) K. Heyne

Nature of allergy	1+		2+		3+		4+ and above		Total reactivity		Total significant reactivity	
	N	%	N	%	N	%	N	%	N	%	N	%
Rhinitis	159	10.6	22	1.47	03	0.20	03	0.20	187	12.47	28	1.87
Asthma	210	14.00	67	4.47	16	1.07	05	0.33	298	19.87	88	5.87
Rhinitis cum Asthma	167	11.13	38	2.53	05	0.33	01	0.07	211	14.07	44	2.93
Total	536	35.73	127	8.47	24	1.60	09	0.60	696	46.40	160	10.67

Table 6. Family history and reaction to *Peltophorum pterocarpum* (DC) K. Heyne

Family history	1+		2+		3+		4+ and above		Total positivity		Total significant positivity	
	N	%	N	%	N	%	N	%	N	%	N	%
- ve	249	16.60	65	4.33	13	0.87	04	0.27	331	22.07	82	5.47
+ ve	287	19.13	62	4.13	11	0.73	05	0.33	365	24.33	78	5.20

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Table 7. Locality and nature of reaction to *Peltophorum pterocarpum* (DC) K. Heyne

Locality	1+		2+		3+		4+ and above		Total positivity		Total significant positivity	
	N	%	N	%	N	%	N	%	N	%	N	%
Highlands	94	6.27	19	1.27	03	0.20	-	-	116	7.73	22	1.47
Midlands	321	21.40	65	4.33	14	0.93	08	0.53	408	27.20	87	5.80
Coastalbelt	121	8.07	43	2.87	07	0.47	01	0.07	172	11.47	51	3.40
Total	536	35.73	127	8.47	24	1.60	09	0.60	696	46.40	160	10.67

patients (35.73%). Among the total positive patients, 127 patients (8.47%) showed 2+ reactivity whereas 24 (1.60%) and 09 (0.60%) patients responded with 3+ and 4+ reactivity as well.

Over all present investigation recorded an average of 46.27% of total reactivity among the patients suffering from respiratory ailments. During late 1990s two independent studies conducted by Prakashkumar among the people of Kerala recorded a total reactivity of 38.4% and 38.8% respectively which is very low when compared with the present results. This also indicate the increased incidence of hypersensitivity during last twenty years in Kerala. Moreover the absence of non-allergic components in the pollen antigenic extract may be one of the possible reasons for the increased skin test results.

Workers like Ahlholm et al. (1998) and Sluyter et al.1998) have already established that skin test results possibly may be influenced by various internal as well as external factors. So a detailed analysis of skin test data was carried out in the light of parameters such as age of the patient, sex, clinical history, family history and the specific locality to which they inhabit.

Age

With regard to Age of patents, (Table: 03), the incidence of total skin test reactivity varies from 09.67% to 12.93%. For age group of 20-29yrs the reactivity recorded was 12.93%, while 09.67% of reactivity was for 10-19 yrs and 11.80% for patients of 40-49yrs. (Table: 04).Results indicates that age of the patients has no significant influence on their skin test reactions, which validates the report by workers like Hanneuseet al (1978), and Prakashkumar (1998). Tsang et al (2000) after a series of investigations stated that it is the internal factors rather than age that influence allergic reactions in man. Anja Mediaty and Karsten (2005) tried to give a proper explanation for this by conducting a series of biochemical studies. Finally it was stated that if a person possess an Ig E level less than 300KU/I, the antibody concentration decline with his age and if the same was greater than 300KU/I, the Ig E concentration remains same. They concluded that the age of an allergic patient become an influencing factor only if he possess comparatively low concentration of Ig E in his blood serum.

Sex

Sex-wise classification of skin test reactivity to *Peltophorum pterocarpum* antigen among 1500 patients with respiratory allergy is illustrated in table 04. From table it is evident that for all reactive groups, male patients reacted more to *Peltophorum pterocarpum* antigen. They showed 20.53% , 5.40%,1.00% and 0.47% reactivity for 1+, 2+, 3+ and 4+ and above group, which make 27.40% (n=411) of the total reactivity. Among the female group this reading is reduced to 19.00 % (n = 285) with individual reactivity of 15.02%, 3.07%, 0.60 and 0.13% for 1+, 2+, 3+ and 4+ and above groups respectively. Szalai (1972) and Broderet al. (1974) attempted to give a proper explanation to this situation. They

concluded that, it is due to our social life pattern in which males frequently go outdoors than female counterparts and get sensitized by different allergic substances. This elicits different forms of allergic reactions among them and this explanation can be applied in the present study also.

Clinical History

Based on the nature of the allergic reactions of the patients reported to Medical College Hospital, Thiruvananthapuram, they were grouped into three major categories such as those with allergic rhinitis, asthma and rhinitis cum asthma. With respect to the significant reactivity it is evident that *Peltophorum pterocarpum* pollen cause significant positive allergic reactions among the asthmatic groups (5.87%) (Tables: 05). Workers such as Shivpuri and Singh (1971), Raju et al. (1990) also made similar observations and recorded high percentage of allergy among patients with clinical history of asthma. Tsai et al (2003) tried to give a proper explanation for this. He identified a specific Ig E in the serum of asthmatic patients which is more reactive and specific than normal Ig E. This may be the reason for the high incidence of reactivity among asthmatic patients.

Family History

The percentage distribution of patients based on the family history of respiratory allergy is represented in table 06. Among the 1500 patients tested with *Peltophorum pterocarpum* pollen antigen, 24.33% (n = 365) of them have a previous family history of allergy. 22.07% (n = 331) have no family history of allergy. For significant positivity also, same trend was observed, i.e. 5.20% and 5.47% respectively. Similar observations was recorded by Wicht and Hugo (1975).They stated that there is no relation between allergy and family history which can be found suitable in the present study as well. Therefore it can be concluded that allergic manifestations among humans are determined by the nature and type of pollen allergens and not the family history, to a great extent.

Locality

In present study, allergic patients, whose hypersensitive activity was studied, hailed from different localities and regions in Kerala. A group of approximately five hundred patients from each locality such as highlands, midlands and coastal belt were selected for the study. The aerobiological survey revealed a higher percentage of distribution of the pollen grains of *Peltophorum pterocarpum* among the midlands of Kerala and similar picture was revealed in the skin test results also (Table 07). When the total significant reactivity was taken into consideration, patients from Midlands showed the maximum reactivity (27.2 %, n= 408). For significant positivity, similar results were observed (5.80%, n = 87). This show that *Peltophorum pterocarpum* pollen elicit more allergic problems to people inhabiting midlands where its aerial presence was also detected high. These results indicate that the presence of a particular pollen type in a given area has significant influence on the allergic conditions of the patients inhabiting in such localities.

Similar finding was reported by other workers also from different parts of the world. Vobrazkova et al (1986) noticed that allergic reactions are highly influenced by the locality where the patients inhabit. Workers like Paul et al (1975) and Seitz et al (2008) reported that the locality in which the patient inhabits has a significant influence in eliciting allergic manifestations among them. Obtulowiczet al (1996) studied the allergic reactions in two localities namely a polluted urban and unpolluted rural area of Poland. They observed that there is a marked increase in allergic reactions among the people belonging to the urban area, irrespective of their family history of allergic diseases. It was also stated that a proper analysis of the aerospora of the surrounding environment will be helpful in designing a proper immunotherapy schedule for the allergic reactions.

Conclusion

The present study has proved that the pollen grains of *Peltophorum pterocarpum* which are aerially dominant in the atmosphere of Kerala state were potent allergens to the human beings. It was observed that, significant skin test positivity is high among male patients. The overall skin test response in this study indicate that the allergic response is independent of the age of the patients. Patients with clinical history of asthma showed maximum reactivity. The skin test results indicate that family history of patients has no significant influence on the skin test positivity. It is also understood that locality of patient had a high influence on eliciting hypersensitive reactions among human beings.So further investigations are needed to reveal the chemical fractions which make this plant species a potent allergen. This will be useful in future to develop better therapeutic methods for managing hypersensitive reactions caused due to these two pollen allergens.

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