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Student's Section

Determination of aerobiological flora associated with allergic rhinitis by Skin Prick Test in a tertiary care hospital in the West Coast of Southern India

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ABSTRACT

Background: Allergic rhinitis (AR) is the most common cause of rhinitis impairing quality of life and increased cost of health care.

Objectives: Primary objective was to determine environmental exposure to pollen allergens of aerobiological flora associated with AR by Skin Prick Test (SPT).

Methods: Bi-directional, cross-sectional study conducted after obtaining approval from Institutional Ethical Committee and Medical Superintendent. Informed consent was obtained in prospectively recruited patients and identity of all retrospective patients was kept anonymous.

Results: From October 2009 to July 2013, 116 subjects enrolled, 69.8% (81) and 30.2% ($n = 35$) from urban area. Mean age was 32.31 years ± 11.51 SD. SPT to any pollen allergens were sensitive in half the cases ($n = 58$) and sensitivity to *Parthenium hysterophorus* was found in 24.1% ($n = 28$). Residents of urban areas were statistically significantly more likely to have a positive SPT to any pollen allergen: [Crude OR (95% CI) 3.02 (1.30–6.97); p -value = 0.001], any non-pollen allergen: [Crude OR (95% CI): 2.84 (1.06–7.64); p value = 0.04], or any allergen: [Crude OR (95% CI): 3.73 (1.03–13.48); p value = 0.045].

Conclusion: Since half the subjects with AR have SPT positive to any aerobiological allergen, and since urban patients are at a higher risk hence efforts must be made to identify the possible allergens and then to develop strategies to avoid them.

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1. Introduction

Allergic rhinitis (AR) is the most common cause of rhinitis, effecting 20–40%.¹ AR results in impaired quality of life and

increased cost of health care. It is an IgE-mediated immunologic response of nasal mucosa to air-borne allergens. AR is characterized by a symptom complex triggered by an immunoglobulin E (IgE)–mediated response to an extrinsic protein. Symptoms of AR consists of any combination of the following:

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sneezing, nasal congestion, nasal itching, and rhinorrhoea.¹ The mucus membranes of eyes, ears, sinuses, and throat can also be involved and get inflamed.

The tendency to develop AR is genetically determined. In susceptible individuals, exposure to certain allergen leads to production of specific IgE directed against these proteins. This specific IgE coats the surface of mast cells, which are present in the nasal mucosa. When the allergen is inhaled into the nose, it can bind to the IgE on the mast cells, leading to immediate and delayed release of a number of mediators like histamine, tryptase, chymase, kinins heparin, leukotrienes and prostaglandin.² These mediators, via various interactions, ultimately lead to the symptoms of AR. This is called the early, or immediate, phase of the reaction. This can be followed by a late phase which may persist for hours or days. Onset of AR is usually in childhood, adolescence, and early adult years, with a mean age of onset 8–11 years, with 80% of cases, AR developing by age 20 years.¹ Prevalence decreases with increasing age.

AR occurs in all races but prevalence varies due to genetic differences, geographic factors or environmental differences, or other population-based factors. In childhood, AR is more common in boys than in girls, but in adulthood, the prevalence is approximately equal between men and women.

Aerobiological flora allergen exposure could be environmental or occupational.¹ These could be insect, dust, animal and fungal allergens, which result in perennial symptoms or to pollen which leads to seasonal symptoms. In a study conducted in Lucknow, Northern India, most common offending allergens were insects (21.2%), followed by dusts (12.0%), pollens (7.8%), animal dander (3.1%), and fungi (1.3%).³

Exposure to allergens can be elicited by history taking and confirmed by Skin Prick Test (SPT).^{1,4} Small amounts of allergen are introduced into the epidermis and non-vascular superficial dermis and interact with specific IgE bound to cutaneous mast cells. Histamine and other mediators are released, leading to a visible “wheal-and-flare” reaction peaking after about 15 min. There has to be a negative control, which is the same solution as the allergens are made in, as well as a positive control, which can be a solution of histamine. A wheal of >3 mm to negative control or <4 mm to positive control would mean that the SPT is un-interpretable.⁴

This study was conducted with the primary objective to determine environmental exposure to pollen allergens of aerobiological flora associated with AR by SPT. The secondary objectives were to determine the proportion of patients of AR in which SPT is negative against the panel of allergens tested, to determine insect, animal dander, dust and fungal allergens of aerobiological flora associated with AR by SPT, and to assess the differences, if any, between aerobiological flora allergens associated with AR as determined by SPT among (a) urban versus rural and (b) male versus female patients.

2. Methodology

2.1. Setting

This study was conducted in the Allergy Clinic, Department of Ear, Nose and Throat (ENT), Kasturba Medical College,

Manipal. The daily attendance of this clinic is 10–15 patients per day of which approximately 0–1 are cases of AR and are referred by the treating doctor for SPT. The study was conducted after obtaining institutional ethical clearance. Thereafter, informed written consent was taken from the participants or their guardians (for those <18 years of age) prior to recruitment. For retrospective cases, permission was taken from the Medical Superintendent of Kasturba Hospital and the cases were recruited from the Medical Registry Department of Kasturba hospital (MRD).

2.2. Design

Bi-directional study, prospective as well as retrospective study.

2.3. Definition

AR was defined as second or subsequent episode of sneezing, nasal congestion, nasal itching, and rhinorrhoea in subjects without fever and after exposure to allergen(s).

2.4. Inclusion criteria

These were (a) Patients with symptoms of AR as defined above (b) >10 years and <60 years of age (c) immunocompetent and (d) consenting for participation. For those between 10 and 18 years of age, in addition to consent taken from the parent or guardian, assent will be taken from the subject for participation.

Case record files of patient of AR who had SPT done, after obtaining informed consent for the procedure, from the MRD which had information on health status, presence or absence of inclusion and exclusion criteria that were similar to those used for prospectively recruited subjects, ENT history and examination findings, SPT Result findings.

2.5. Exclusion criteria

These were (a) presence of Dermatographism (b) history of use of Antihistamines, tetracycline, antidepressants (tricyclic), phenothiazine, over the counter cough and cold medications, anti-emetics, analgesics, migraine prophylactic drugs and sedatives for the past week (c) use of Aspirin in the past week (d) pregnancy (e) known cases of congenital or acquired immunodeficiency.

In all retrospective cases, the aforementioned exclusion criteria were matched with the detailed history in the files of all the subjects who underwent a SPT in the past.

2.6. Sampling technique

Consecutive patients of AR who fulfilled the inclusion criteria and had no exclusion criteria and who had been referred for SPT by the treating doctor were invited to participate in the study. Clinical data was collected by interview of the patient and abstracted from hospital records on a preformed questionnaire in the prospective study. SPT was performed on all those included by a trained nurse who was posted in the

allergy clinic. Data from medical records was abstracted from the MRD.

2.7. Skin prick testing (SPT)

Subjects were asked to expose their forearm. The forearm was cleaned by cotton and spirit. First, Saline (0.9%) was inoculated which is negative control via a single lancet prick (no false positive results) ensuring that no blood oozed out; second was Histamine (0.1%), the positive control; Then the allergen(s) to be tested were inoculated based on the allergen chart provided. Wheal and flare was observed and recorded after 20 min. The base value of wheal and flare surrounding the allergen, negative and positive control was recorded in mm. To be called positive reaction with the supposed allergen the area of wheal and flare should be ≥ 2 mm to that of the area of wheal and flare recorded in the case of the saline inoculum.

2.7.1. Negative SPT

Subjects who did not react with any allergen other than the positive and negative control. Monosensitive: Subjects who were sensitive to only one tested allergen. Oligosensitive: Subjects who were sensitive to 2 tested allergens. Poly-sensitive: Subjects who were sensitive to 3 or more allergens. If more than 5 allergens were found positive then prescription for allergic treatment was given by the treating medical doctor.

The SPT was done against the following 22 allergens. The SPT was done using commercially available kits manufactured by Creative Diagnostic Medicare Pvt. Ltd., D-296, Vashi Plaza, Sector 17, Vashi, Navi Mumbai. This kit is being currently used in the allergy clinic viz:

1. Pollen: (*Chenopodium*, *Ricinus communis*, *Brassica nigra*, *Parthenium*, *Cassia-Siamea*, *Eucalyptus*)
2. Mite: (House mite)
3. Insect: (Cockroach male & female, Ants, Mosquitoes)
4. Animal: (Dog dander)
5. Dust: (House, Cotton mill, Wheat grain, Paper)
6. Fungus: (*Aspergillus fumigatus*, *Candida albicans*, *Alternaria tenuis*, *Aspergillus niger*, *Aspergillus tumari*, *Culvularia*)

In the subjects recruited retrospectively, the test kit remained the same.

2.8. Variables for data collection

Data was collected on pre-designed, pilot tested questionnaires. Data was collected on demography (age, sex, place of residence); present history of rhinitis, congestion, nasal itching with duration, as well as past history of similar episodes with their relationship to seasons. History was taken for presence of eczema, asthma, allergic rhinitis, and allergic conjunctivitis in the patient or their family. Data of clinical examination of the nose (deviated nasal septum, polyp, status of turbinates), eyes (conjunctivitis, congestion), respiratory system examination (ronchi/crepitations) was recorded. Deviated Nasal Septum (DNS) was considered as present if there was deviation of septum to either side with or without a spur. Nasal Polyp was considered as present when a

polypoidal mass which on probing being hypo-aesthetic and non-friable was seen through either anterior or posterior rhinoscopy. Mucosa was considered as having a pathology when it was congested, pale, oedematous or showing polypoidal changes. Turbinate was considered as having a pathology when there was turbinate hypertrophy, oedema, or associated polypoidal changes over the turbinate. Any mucoid discharge was considered as positive for discharge.

2.9. Sample size

Based on a study done in Lucknow, we have assumed that 7.8% AR will be due to exposure to pollen allergens. To detect this with a precision of 5% and with an alpha value of 5%, the minimum sample size to be recruited will be 111 patients of AR.

2.10. Data management and analysis

Data was entered in MS Excel. Frequency of all variables was assessed. We are reporting the proportion (with 95% confidence interval) positive for (a) any allergen, (b) pollen and (c) other allergens and (d) negative for all tested aerobiological allergens. We have compared SPT positivity between rural and urban patients and also between males and females by calculating the crude odd's ratio and 95% confidence interval with *p* vales (using a 2-tailed distribution). We have compared the baseline characteristics of those positive to pollens alone versus those positive to any other allergen using chi square test for categorical and student's *t* test for continuous variables. Using a 2-tailed distribution a *p* value of <0.05 has been taken as statistically significant.

3. Results

Data was abstracted from hospital records of 111 patients from July 2013 to October 2009, those who fulfilled all inclusion criteria and had no exclusion criteria. The identity of retrospective subjects was kept anonymous by the hospital record section. 5 patients were recruited prospectively from April 2013 to July 2013. Amongst the 116 subjects enrolled, 56% (*n* = 65) were male. Urban residents accounted for 30.2% (*n* = 35) whereas 69.8% (*n* = 81) were from rural areas. Mean age of the subjects enrolled was 32.31 years $\pm$ 11.51 SD.

Family history of nasal allergy was present in 17.2% (*n* = 20) subjects and Bronchial Asthma in 13.8 (*n* = 16) subjects. The clinical symptoms on presentation along with their duration are given in Table 1. Findings of clinical examinations are also given in Table 1. The most prevalent presenting symptom was rhinorrhoea (87.1%). Red eyes had the least prevalence as a presenting symptom (1.7%). 47 subjects came with the triad of symptoms (40.5%) i.e. Rhinorrhoea, Blocked nose and Epiphora. On clinical examination, DNS was the most common deformity seen (70.7%).

In Table 2, the SPT positivity to various allergens has been given. Amongst the pollen found to have triggered an allergic response, Parthenium was found to have been implicated in most of the subjects, 24.1% (*n* = 28). It was found that SPT positivity to Parthenium was statistically significantly higher

Table 1 – Clinical symptoms and examination findings of enrolled patients with Allergic rhinitis.

		n	%
Clinical symptoms			
Nose	Running nose	101	87.1
	Blocked nose	85	73.3
	Itchy nose	7	6
Eye	Watering	56	49.1
	Red eyes	2	1.7
Skin	Skin eczema	4	3.4
Respiratory system	Breathlessness	3	2.6
Clinical examination			
Systemic	Pallor	8	6.9
Nose	DNS	82	70.7
	Polyp	12	10.3
	Mucosa	50	43.1
	Turbinate	57	49.1
	Discharge	43	37.1
Eye	Conjunctival congestion	2	1.7
	Transverse crease	4	3.4
Respiratory auscultation	Crepitations or ronchi	2	1.7
Abbreviation: DNS = Deviated nasal symptoms.			

in the urban population as compared to the rural population [40% (14/35) versus 17.31% (14/81); p value = 0.01]. Amongst non-pollen allergen, male cockroach was found to be the most common allergen in 26.7% (n = 31) subjects and least to *A. niger* in 3.4% (n = 4) subjects.

In Table 3, categorical distribution of aerobiological allergens in patients with AR has been reported. Also, comparison has been reported between proportions positive for SPT to various allergens in male versus female and urban versus rural subjects. It was found that subjects coming from urban

Table 2 – Sensitivity to individual aerobiological allergens.

	n	%
Chenopodium	20	17.2
Ricinus communis	13	11.2
Brassica nigra	15	12.9
Parthenium	28	24.1
Cassiasiaamea	16	13.8
Eucalyptus	15	12.9
House mite	12	10.3
Cockroach male	31	26.7
Cockroach female	21	18.1
Ants	23	19.8
Mosquito	20	17.2
Dog dander	9	7.7
House dust	23	19.8
Cotton mill dust	22	19
Wheat grain dust	16	13.8
Paper dust	13	11.2
<i>Aspergillus fumigatus</i>	17	14.7
<i>Candida albicans</i>	7	6
<i>Alternaria tenuis</i>	13	11.2
<i>Aspergillus niger</i>	4	3.4
<i>Aspergillus tumari</i>	8	6.9
Culvularia	11	9.5

areas had a statistically significant SPT positive reaction to Pollen, Dust and Fungi as compared to the rural population (Table 3). Residents of urban areas were statistically significantly more likely to have a positive SPT to any pollen allergen: [Crude OR (95% CI) 3.02 (1.30–6.97); p -value = 0.001], any non-pollen allergen: [Crude OR (95% CI): 2.84 (1.06–7.64); p value = 0.04], or any allergen: [Crude OR (95% CI): 3.73 (1.03–13.48); p value = 0.045] tested at the allergic clinic.

4. Discussion

This study was conducted in the West Coast of India to find the pollen allergens of aerobiological flora associated with AR as determined by SPT. Half the subjects with AR had SPT positivity for any pollen allergen. Statistically higher proportion of subjects from urban area were sensitive to pollen, dust, and fungal allergens as compared to those from rural areas. It was also found that statistically significant higher proportion of male subjects were SPT positive for insect and mite allergens.

Parthenium hysterophorus is a species of flowering plant belonging to the family Asteraceae that is a common invasive weed found in India. Contact with this plant can manifest as dermatitis and respiratory malfunction in humans. *Parthenium* exerts its effects through toxins like parthenin, ambrosin, and others.⁵

In this study it was found that SPT positivity to *P. hysterophorus* was found in almost half of the 54 patients allergic to any pollen allergens. These findings are similar to those reported in a study conducted in Karnataka, India, which also concluded that the most common allergen was *Parthenium*.⁶ *Chenopodium* was the second most common pollen to cause a positive reaction in the SPT (17.2%). This is in contrast to a study conducted in New Delhi which concluded that *Chenopodium* was found to be sensitive in only 1.53% of cases.⁷

In this study it was found that male cockroach was the most common allergen (31%). In the current study, in subjects with AR, SPT was positive for Pollens (50%) were followed by Dust (42.2%), Insects (38.8%), Fungi (34.5%), Mites (10.3%), and Dog Dander (7.7%). On comparing these findings with a study conducted in Lucknow, a city in Northern India, unlike the present study, the most common affecting allergens were insects (21.2%), followed by dust (12.0%), pollens (7.8%), animal dander (3.1%), and fungi (1.3%).³ Another study conducted in New Delhi showed the prevalence of sensitivity to SPT to be 43.9% in insects, weed pollen (21.79%) and tree pollen (15.14%), dust (14.49%), house mite (12.42%), and fungi (11.98%). In the same study conducted in Delhi, it was concluded amongst the insects tested, mosquitoes showed a sensitivity in 31.92% sensitivity; amongst the fungi tested, *A. fumigatus* and *Candida* were positive in 4.25% and 1.53% subjects respectively.⁷ In the present study, unlike the reported finding from the Delhi study,⁷ only 7.2% of subjects were sensitive to mosquitoes, 14.7% were sensitive to *A. fumigatus* and 6% to *Candida*, which depicts a lower sensitivity to mosquitoes and a higher sensitivity to fungi. The study area is endemic to mosquitoes. Unlike the findings in the present study, a study in Gujarat, India, concluded that Insects were the predominant allergen in SPT.⁸ A study from Jordan reported that 32.9% of

Table 3 – Categorical distribution of aerobiological allergens in patients with allergic rhinitis.

	n	%	Urban (35) n, %	Rural (81) n, %	p value (urban vs. rural)	Male (65) n, %	Female (51) n, %	p value (male vs. female)
Pollen	58	50	24, 68.6	34, 42	0.009	32, 49.2	26, 51	NS
Mite	12	10.3	6, 17.1	6, 7.4	NS	10, 15.4	2, 3.9	0.04
Insects	45	38.8	16, 45.7	29, 35.8	NS	28, 43	17, 33.3	0.02
Dog dander	9	7.7	4, 11.4	5, 6.2	NS	3, 4.6	6, 11.6	NS
Dust	49	42.2	21, 60	28, 34.6	0.01	28, 43	21, 41.2	NS
Fungi	40	34.5	21, 60	19, 23.5	0.000	24, 36.9	16, 31.4	NS

Table 4 – Number of subjects with significant allergy and those who were negative reactors to Skin Prick Test.

	N	%	Urban (35) n, %	Rural (81) n, %	Male (65) n, %	Female (51) n, %
Significant allergy	38	32.8	21, 60	17, 21	23, 35.4	15, 29.4
Negative SPT	24	20.9	3, 8.6	21, 25.9	14, 21.5	10, 19.6
Monosensitive	23	19.8	7, 30.4	16, 69.6	13, 56.5	10, 43.5
Oligosensitive	16	13.8	1, 6.3	15, 93.8	5, 31.3	11, 68.8
Polysensitive	53	45.7	24, 45.3	29, 54.7	33, 62.3	20, 37.7

the subjects of AR showed a sensitivity to Mites⁹ which is higher than the proportion found in the current study. A study in Greece showed that 77.8% subjects with AR were sensitive to Pollen and 43.2% were sensitive to mites¹⁰ both of which are higher than the findings of the current study. Also, unlike the current study, a research conducted in the Iberian Peninsula has reported that the most common allergens are Mites and grass pollen.¹¹

In the current study it was found that 19.8% were monosensitive, 13.8% oligosensitive, and 45.7% were polysensitive to SPT (Table 4). A study conducted in Iran showed that 81% of the test population was sensitized and 76% of the test population was polysensitized to various aerobiological allergens¹² in patients with AR, which is higher than the results reported in the current study. In contrast to the current study, the study in Greece concluded that 12.4% of subjects having undergone SPT were monosensitive, 58.7% were oligosensitive and 28.9% were polysensitive.¹⁰ Thus it is obvious that there is a geographical variation in the patterns of aerobiological sensitivity in patients with AR and site specific data are necessary for effective management.

The strength of this study was that the subjects had well defined AR. However, subjects were recruited prospectively as well as retrospectively and this could have resulted in measurement bias even though the same kits were used for SPT. Also this was a cross sectional study and subjects were not followed up to assess the impact of avoidance of allergen on symptoms of AR. Recently specific IgE against allergens is measured to identify the possible cause of AR but this was not planned for the current work and hence not done.

In subjects with AR attending a tertiary care centre in the West Coast of Southern India, SPT was positive for pollen allergens in half the cases, and specifically it was due to *P. hysterophorus*. Male cockroach was the most common allergen in this setting. Subjects with AR from urban areas were at greater risk for having SPT positivity for any allergen. A study conducted in Mysore, India, concluded that children from rural vs urban areas had a lower prevalence of self-reported asthma (8% vs 30%), rhinitis (22% vs 42%), and atopic

sensitization (36% vs 58%).¹³ Hence, efforts in these must be made to first identify the possible allergens and then to develop strategies to avoid them.

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Conflicts of interest

All authors have none to declare.

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