

Allergenic pollen in the atmosphere of Rohtak city, Haryana (India): a pioneer study

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Abstract An attempt has been made in the present study to assess the allergenicity of dominant pollen types recorded from the atmosphere of Rohtak city. Skin prick test was performed with the antigenic extracts of 22 pollen types on 150 local patients who visited Asthma Clinic, University of Health Sciences, Rohtak. Markedly positive skin reactions (2+ and above) varied from 4.6 to 20.6 % to various pollen antigens. *Cenchrus ciliaria* (20.6 %), *Zea mays* (20 %) and *Pennisetum typhoides* (19.3 %) were the pollen allergens exhibiting maximum sensitivity. Antigenic extract of *Cassia occidentalis*, *Cynodon dactylon* and *Ricinus communis* showed marked skin reactivity in 18.6 % of patients. *Prosopis juliflora*, *Chenopodium murale*, *Amaranthus spinosus*, *Cassia fistula* and *Cassia siamea* showed 2+ and above reactions in 16.6, 15.3, 14.6 and 14.0 % of the local patients, respectively. Least reactivity (4.6 %) was shown to the antigenic extract of *Cyperus rotundus*. Out of 52 sera screened for the presence of specific IgE antibodies against different antigenic extracts, only 5.5 % showed >60 % binding. About 30 % and above binding was shown to the antigenic extracts of *Z.*

mays, *A. spinosus*, *R. communis* and *Xanthium strumarium*. The concordance between positive skin reaction and serum-specific IgE antibodies ranged from 15 to 69 %.

Keywords Pollen · Aeroallergens · Bioassay · Immunoassay

1 Introduction

Air is a repository of an invisible biomass of particulate matter of plant and animal origin. Of these, pollen grains constitute a significant fraction and represent an inevitable allergen source due to their ubiquitous presence in nature (Hyde 1969; Cosentino et al. 1995; Puc 2003). During the last few decades, there has been an increase in the incidence of pollen allergy worldwide, including Asian countries (Bunnag et al. 1997; Wong et al. 2004; Wan-Ishlah and Gendeh 2005; D'Amato et al. 2007). In India also, an increase in allergic diseases from 10 to 30 % has been reported in the last 40 years (Vishwanathan 1964; Chhabra et al. 1998; Gaur et al. 2004). The escalating trends in these diseases are mainly attributed to increased environmental pollution due to industrialization and urbanization and changing lifestyle (Singh and Kumar 2003; Sharma et al. 2009).

India with divergent geoclimatic zones showing marked fluctuation in climatic conditions such as

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temperature and relative humidity has wide and varied floral spectrum. The sources and nature of pollen allergens thus vary in different parts of the country. It is therefore imperative to collect information on pollen allergens of different regions for management of allergic ailments of local population. A great deal of work has been carried out at different eco-geographical regions in India with regard to allergenic pollen flora (Kasliwal et al. 1959; Shivpuri et al. 1979; Gupta et al. 1984; Shivpuri and Parkash 1967; Karmakar et al. 1994; Rawat et al. 2004; Sharma et al. 2009; Wani et al. 2010). However, no such data are available from Haryana, a northern state of India as it has not been explored for pollen allergens. This is also evident from the scarcity of published literature from this area.

Efforts have been made by the authors to initiate the work in this direction. As information on the prevalence of airborne pollen types is useful in the management of pollinosis, aerial pollen spectrum of Rohtak city (Fig. 1), Haryana was investigated at two sites for two consecutive years (July 2007–June 2009). Site I was located in a densely populated area of the city with poor vegetation cover, whereas site II was on the outskirts of the city with luxuriant vegetation in nearby vacant lands and cultivated fields. As a result, 31 pollen types belong to 23 angiospermic families were identified (Ahlawat et al. 2013). The present investigation was envisaged to gain an understanding of allergenicity of 22 pollen types using *in vitro* skin prick test (SPT) and *in vivo* enzyme-linked immuno-sorbent assay (ELISA) tests. Selection of the pollen types was done on the basis of their commercial availability and reported allergenicity. The information obtained will be of paramount importance as it can be used as a valuable tool in the diagnosis and treatment for local patients suffering from nasobronchial allergy.

2 Materials and methods

2.1 Antigen procurement

A total of 22 pollen types prevalent in the airspora of Rohtak city were selected for assessing their allergenicity among local patients. Sterilized antigenic extracts along with 50 % glycerinated phosphate buffer (1:10) and histamine dichloride (1 mg/ml) were procured from All Cure Pharmaceutical Company, Bahadurgarh, Haryana.

2.2 Skin prick test (SPT)

2.2.1 Selection of patients

A total of 150 patients with nasobronchial symptoms attending Asthma Clinic, P.G.I.M.S., University of Health Sciences, Rohtak, Haryana during 2009–2010 were selected for SPT. The selection of patients was based on prior physician diagnosis and a positive bronchoprovocation test. Patients selected for the study were advised not to take antihistamines and tranquilizers (at least 48 h) and other sympathomimetic drugs (at least 18–12 h) before the test. It was also ensured that patients selected have not received corticosteroids for last 3 weeks. Prior informed written consent for performing SPT was obtained from all the subjects. Confidentiality of the patients who underwent the tests was maintained. SPT was performed by a trained paramedic under the supervision of a senior doctor. The study was approved by the Ethical Committee of the concerned institute.

2.2.2 Inclusion/exclusion criteria

Patients in the age group of 18–60 years with a history of respiratory allergy were included for the test. Patients were excluded from the study if they were found to be pregnant, on antihistamines or daily steroids. Other exclusion criteria included dermatophagism, generalized eczema, history of severe allergy or anaphylaxis and chronic debilitating diseases.

2.2.3 Procedure of SPT

The patient was comfortably seated and the volar surface of the forearm was cleaned with 70 % alcohol. A small drop of antigenic extract was placed on the forearm, and a disposable hypodermic syringe (26 G) was inserted through the extract. The needle was held at 45° to the skin, bevel upwards, and the point was inserted about 0.5 mm. The needle was then lifted slightly so that there is tenting of the skin to allow adequate entry of the antigen beneath the epidermis. Similarly, other antigenic extracts were also tested on the patient. Glycerinated phosphate buffer (50 %) and histamine dichloride (1 mg/ml) were taken as negative and positive control, respectively. SPT was also performed on ten healthy non-atopic volunteers to

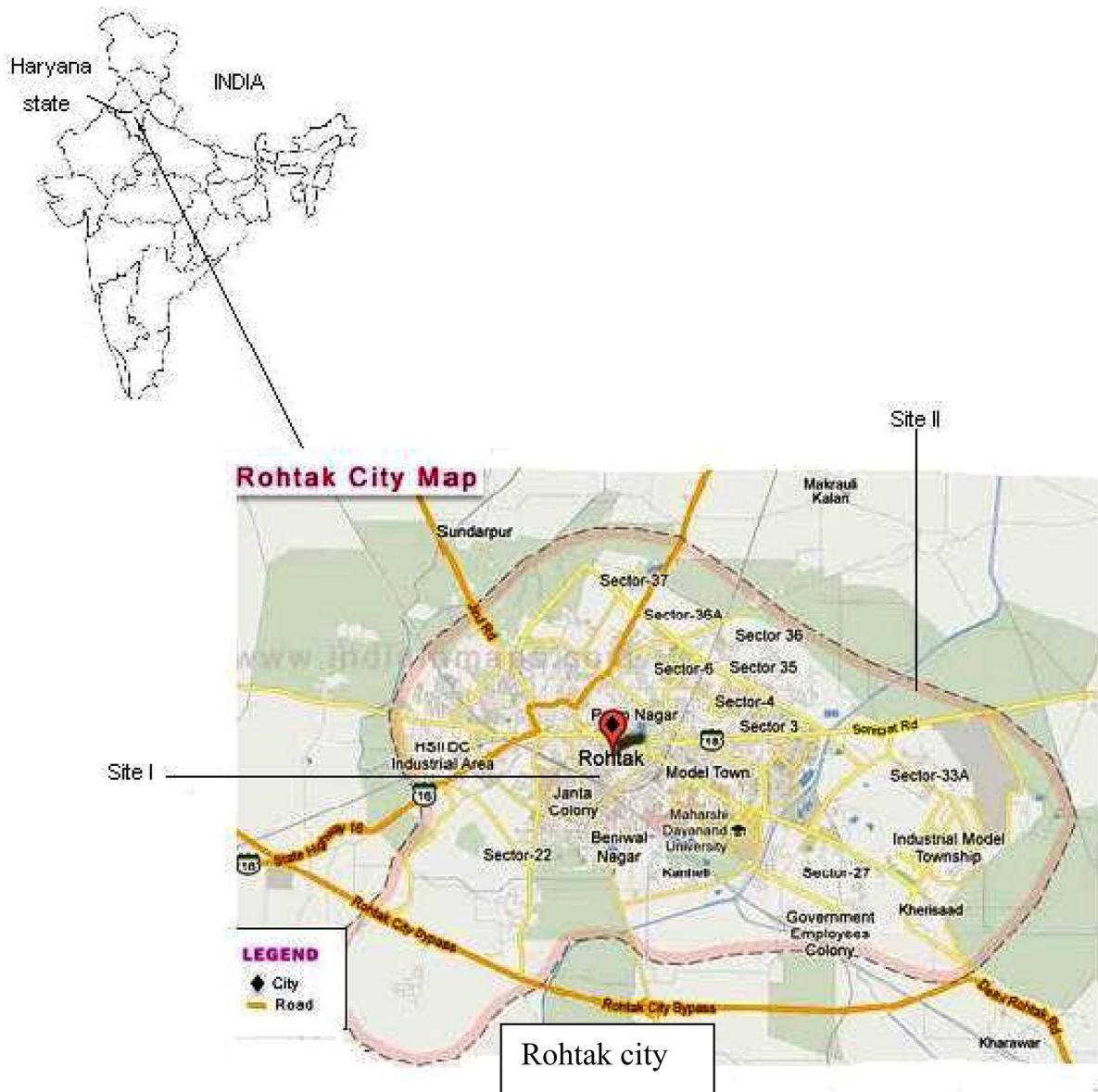


Fig. 1 Location of two pollen monitoring sites in Rohtak city, Haryana

ensure that each antigenic extract was devoid of non-specific irritants.

2.2.4 Grading of SPT

The skin reactions were allowed to develop for 15–20 min, and their intensity was measured with

respect to the wheal and erythema produced. The wheal size was measured with a reaction gauge, and pseudopodia and erythema (if appeared) were also noted. The SPT results were graded as compared to negative and positive control as per the criteria given by Shivpuri (1962): wheal size same as negative control = negative; wheal size 2–3 mm = 1+; wheal

size 4–5 mm = 2+; wheal size 5–8 mm = 3+; wheal size >8 mm = 4+.

2.3 Estimation of protein

The soluble protein content of the antigenic extracts was estimated by Lowry's method with slight modification (Lowry et al. 1951). The soluble proteins were precipitated using 10 % phosphotungstic acid, and the precipitate was dissolved in 2 ml of 2 % sodium hydroxide solution. An aliquot was taken, and protein was estimated with the help of a standard curve prepared using serial dilutions of BSA (0–100 µg/ml) against OD values at 660 nm.

2.4 Enzyme-linked immuno-sorbent assay (ELISA)

2.4.1 Serum samples

Blood samples were collected from patients showing markedly positive skin reactions to any one of the pollen antigenic extracts after obtaining their written consent. Blood was allowed to clot for about 1 h at room temperature, and expressed serum was separated. Sera obtained were stored at –20 °C for future use. Blood was also collected from 5 normal individuals who had no history of respiratory allergy and showed no skin reactivity to the antigenic extracts. Sera obtained from these volunteers were used as control.

2.4.2 Procedure for ELISA

Enzyme-linked immuno-sorbent assay was performed as per the procedure outlined by Sepulveda et al. (1979). Polystyrene microtitre plates (Tarsons, 96 well) were coated with 100 µl of pollen antigen (200 µg protein/ml) and incubated overnight at 4 °C. Plates were thoroughly washed with 0.05 M phosphate buffer saline, and 100 µl of 1 % BSA was added to block the unbound sites. After washing, 100 µl of patient's sera (diluted to 1:10 with PBS containing 0.05 % BSA) was added to each well and incubated overnight at 4 °C. After repeated washing, each well was incubated with 100 µl antihuman IgE alkaline phosphatase (Sigma) diluted to 1:1000 at 37 °C for 4 h. About 100 µl substrate p-nitrophenol phosphate

(PNPP) (1 mg/ml) was added to each well for colour development. Reaction was terminated after 45 min with 5 N sodium hydroxide solution. OD value was measured at 405 nm using ELISA reader (Thermo Scientific Multiskan Ascent). Serum from normal volunteers was also analysed against all antigenic extracts. Depending upon percent binding, OD values obtained were classified into four groups as per the criteria of Kauffman et al. 1983 (>15 %, 15–30 %, 30–60 % and <60 %).

Calculation of Percent Binding:

$$\frac{A_o - A_y}{A_x - A_y} \times 100$$

where A_o , Absorbance of individual sera; A_x , Mean maximum absorbance obtained with 10 highly positive sera; A_y , Mean absorbance obtained with normal sera.

3 Results

3.1 Bioassay

In the present investigation, a total of 3,300 skin tests were performed on 150 patients with the antigenic extracts of 22 pollen types. The skin reactions were classified as positive, markedly positive and highly positive and are summarized in Table 1. Patients showing more than 2+ reactions were considered as markedly positive, and those showing 3+ and above reactions were considered as highly positive. Out of 150 patients, 49.4 % subjects showed 1+ and above skin reaction towards all antigenic extracts. The percent of patients showing markedly positive skin reactions to various pollen antigens varied from 4.6 to 20.6 %. High skin reactivity was shown towards antigenic extracts of *Cenchrus ciliaris* (20.6 %) followed by *Zea mays* (20 %) and *Pennisetum typhoides* (19.3 %). Antigenic extract of *Cassia occidentalis*, *Cynodon dactylon* and *Ricinus communis* exhibited marked skin reactivity in 18.6 % of patients. *Prosopis juliflora*, *Chenopodium murale*, *Amaranthus spinosus*, *Cassia fistula* and *Cassia siamea* showed 2+ and above reactions in 16.6, 15.3, 14.6 and 14.0 % of the subjects, respectively. Among all the antigenic extracts used for SPT, *Cyperus rotundus* showed least reactivity (4.6 %).

Table 1 Number and percentage of patients showing positive, markedly positive and highly positive skin reactions to different antigenic extracts

Sr.No.	Pollen antigens	Positive (1+ to 4+)		Markedly positive (2+ to 4+)		Highly positive (3+ and above)	
		(%)	No.	(%)	No.	(%)	No.
1.	<i>Amaranthus spinosus</i>	15.3	23	14.6	22	6	09
2.	<i>Artemisia scoparia</i>	11.3	17	8	12	2.6	04
3.	<i>Azadirachta indica</i>	11.3	17	9.3	14	2.6	04
4.	<i>Cannabis sativa</i>	12	18	11.3	17	3.3	05
5.	<i>Cassia fistula</i>	12.6	19	14	21	3.3	05
6.	<i>Cassia occidentalis</i>	17.3	26	18.6	28	6.6	10
7.	<i>Cassia siamea</i>	16	24	14	21	5.3	08
8.	<i>Cenchrus ciliaria</i>	21.3	32	20.6	31	8.6	13
9.	<i>Chenopodium album</i>	13.3	20	12.6	19	5.3	08
10.	<i>Chenopodium murale</i>	15.3	23	15.3	23	5.3	08
11.	<i>Cynodon dactylon</i>	18	27	18.6	28	6.6	10
12.	<i>Cyperus rotundus</i>	4.6	07	4.6	07	1.3	02
13.	<i>Eucalyptus tereticornis</i>	9.3	14	8.6	13	3.3	05
14.	<i>Impertea cylindrica</i>	12	18	12.6	19	4.6	07
15.	<i>Morus alba</i>	10	15	10	15	4	06
16.	<i>Parthenium hysterophorus</i>	11.3	17	11.3	17	4.6	07
17.	<i>Pennisetum typhoides</i>	18	27	19.3	29	8.6	13
18.	<i>Prosopis juliflora</i>	16.6	25	16.6	25	6.6	10
19.	<i>Ricinus communis</i>	19.3	29	18.6	28	7.3	11
20.	<i>Sorghum vulgare</i>	11.3	17	10	15	3.3	05
21.	<i>Xanthium strumarium</i>	14	21	13.3	20	4	06
22.	<i>Zea mays</i>	20.6	31	20	30	9.3	14

Total number of patients tested: 150

3.2 Immunoassay

Out of the 74 patients showing markedly positive skin reactions, only 52 consented for the in vitro study. Their sera were therefore screened for the presence of specific IgE antibodies against antigenic extracts to which they showed positive skin reactions. The mean value of OD obtained both for patients and control sera against each pollen antigen is listed in Table 2. Sera showing percent binding >15 % were considered as positive, and those showing more than 30 % binding were considered as highly positive for ELISA. Out of the total sera tested, 66.4 % were considered negative as they showed <15 % binding with the antigenic extracts. Only 5.5 % of the patient's sera tested showed >60 % binding and rest showed binding between 15 and 60 %. Higher grade of IgE binding (30 % and above) was shown to the antigenic extracts

of *Z. mays*, *A. spinosus*, *R. communis* and *Xanthium strumarium* (Table 3).

3.3 Percent correlation between SPT and ELISA

Percent correlation between SPT and ELISA was found to vary from 15 to 69.2 % (Table 4). Greater than 50 % correlation was observed for *Artemisia scoparia*, *C. fistula*, *R. communis* and *Z. mays*. However, it was less for the pollen extracts of *C. siamea*, *P. typhoides*, *C. ciliaria*, *C. dactylon* and *C. rotundus*.

4 Discussion

Identifying aeroallergens of different geographical regions has been the main aim of several studies

Table 2 Mean values of O.D. shown by patient's sera and control for each antigenic extract

Antigens	No. of patients tested for ELISA	Mean O.D.	
		Patient	Control
<i>Amaranthus spinosus</i>	21	0.80	0.07
<i>Artemisia scoparia</i>	12	0.75	0.07
<i>Azadirachta indica</i>	14	0.69	0.04
<i>Cannabis sativa</i>	17	0.40	0.03
<i>Cassia fistula</i>	13	0.37	0.03
<i>Cassia occidentalis</i>	22	0.35	0.02
<i>Cassia siamea</i>	20	0.40	0.04
<i>Cenchrus ciliaria</i>	11	0.39	0.03
<i>Chenopodium album</i>	15	0.44	0.03
<i>Chenopodium murale</i>	19	0.81	0.08
<i>Cynodon dactylon</i>	22	0.30	0.03
<i>Cyperus rotundus</i>	04	0.68	0.05
<i>Eucalyptus tereticornis</i>	11	0.21	0.03
<i>Impertea cylindrica</i>	13	0.41	0.04
<i>Morus alba</i>	14	0.60	0.05
<i>Parthenium hysterophorus</i>	12	0.41	0.03
<i>Pennisetum typhoides</i>	23	0.57	0.04
<i>Prosopis juliflora</i>	24	0.69	0.07
<i>Ricinus communis</i>	21	0.78	0.05
<i>Sorghum vulgare</i>	13	0.25	0.02
<i>Xanthium strumarium</i>	17	0.15	0.04
<i>Zea mays</i>	27	0.70	0.08

carried out throughout the world for management of allergic ailments. This information is of immense importance as it helps the allergologists in selection of antigen for diagnosis and treatment for local patients suffering from allergic ailments. Such studies also gain momentum as aeroallergens and particularly pollen varies greatly depending upon geo-climatic conditions, and allergens used for SPT or specific allergen immunotherapy should be selected from those that are encountered in the patient's geographical area. Therefore, an attempt was made to identify the pollen allergens of Rohtak city. In the present investigation, skin prick method was adopted for bioassay as it is considered safe, less painful and gives more reliable results. Moreover, this methodology is widely used throughout the world to find out the sensitization in hypersensitive patients with allergic ailments (Singh et al. 1987; Wan-Ishlah and Gendeh

2005; Mandal et al. 2008; Fereidouni et al. 2009; Wani et al. 2010).

The percentage of patients showing markedly positive skin reactions to 22 antigenic extracts varied from 4.6 to 21.3 % with an average of 13.7 %. The overall incidence of SPT reactivity was highest against the antigenic extracts of pollen belonging to Poaceae family such as *C. ciliaria*, *Z. mays*, *P. typhoides* and *C. dactylon*. High prevalence of grass pollen allergy is also reported from different parts of the world (Shivpuri et al. 1979; Singh et al. 1987; Stam and Timmermans 1989; Hirsch et al. 2000; Erbas et al. 2007; Mandal et al. 2008). In compliance to our study, *Cynodon* sp., *Imperata* sp. and *Pennisetum* sp. have been reported to be common aeroallergens from a nearby station, Delhi (Dua and Shivpuri 1962; Shivpuri et al. 1979; Singh et al. 1987; Rajkumar 2003). It is interesting to note that high allergenic response against pollen of Poaceae family also commensurates with their high aerial prevalence (20.65 %) in the airspora of Rohtak city (Ahlawat et al. 2013).

Chenopodium murale, *C. album* and *A. spinosus* were also among important allergens eliciting skin reactivity in 15.3, 12.6 and 14.6 % of the subjects, respectively. From Delhi, *A. spinosus* has been shown to be predominant allergen (Singh and Dahiya 2002). Sharma et al. (2009) also reported high positivity (23.5 %) against the antigenic extract of this pollen from Assam. *R. communis* and *P. juliflora* were also important pollen allergens as they showed high incidence of positivity among local patients. From Delhi, Shivpuri and Parkash (1967) reported *P. juliflora* to be a major source of pollinosis with 12 % patients showing a positive skin reaction to its pollen extract. *P. juliflora* and *R. communis* are also reported as predominant aeroallergens by other workers from the same region (Shivpuri et al. 1979; Singh et al. 1987; Dahiya and Singh 2003; Rawat et al. 2004). Studies carried out at Lucknow also showed *P. juliflora* to be a potent allergen (Agnihotri and Singh 1971; Prasad et al. 2009).

In our study, low incidence of positivity was registered against the antigenic extracts of *C. rotundus* and *Eucalyptus tereticornis*. Similar to our findings, *Eucalyptus* sp. did not show any markedly positive skin reaction among the patients of nasobronchial allergy from other parts of the country (Agnihotri and Singh 1971; Prasad et al. 2009).

Table 3 Percent binding of specific IgE against 22 pollen antigens in patients showing markedly positive skin reactions

Antigen used	No. of SPT +ve sera tested for ELISA	Percent Binding			
		0–15 %	15–30 %	30–60 %	>60 %
<i>Amaranthus spinosus</i>	21	13	2	4	2
<i>Artemisia scoparia</i>	12	07	3	1	1
<i>Azadirachta indica</i>	14	08	3	2	1
<i>Cannabis sativa</i>	17	13	2	1	1
<i>Cassia fistula</i>	13	07	3	2	1
<i>Cassia occidentalis</i>	22	18	3	1	0
<i>Cassia siamea</i>	20	17	2	1	0
<i>Cenchrus ciliaria</i>	11	08	2	1	0
<i>Chenopodium album</i>	15	11	2	2	0
<i>Chenopodium murale</i>	19	14	1	2	2
<i>Cynodon dactylon</i>	22	17	2	1	0
<i>Cyperus rotundus</i>	04	03	1	0	0
<i>Eucalyptus tereticornis</i>	11	07	1	2	1
<i>Impertea cylindrica</i>	13	08	3	2	0
<i>Morus alba</i>	14	09	3	1	1
<i>Parthenium hysterophorus</i>	12	08	2	2	0
<i>Pennisetum typhoides</i>	23	19	2	1	1
<i>Prosopis juliflora</i>	24	18	2	3	1
<i>Ricinus communis</i>	21	08	7	3	3
<i>Sorghum vulgare</i>	13	07	3	2	1
<i>Xanthium strumarium</i>	17	07	3	4	1
<i>Zea mays</i>	27	13	6	5	3

On reviewing the pattern of allergenicity exhibited by antigenic extracts of pollen types prevalent in the atmosphere of Rohtak city, it was observed that dominance of the pollen in airspora does not correlate with the level of skin sensitivity observed in patients exposed to these allergens. This is evident by the fact that pollen of *R. communis*, *P. juliflora* and *Cassia* sp. is not among the dominant types encountered in the present study, but they registered high incidence of allergenicity (12.6–19.3 %). On the other hand, aerial prevalence of pollen of *Morus alba* and *Parthenium hysterophorus* was quiet high, but they registered moderate skin reactivity. Similar observations have also been made from other parts of the country (Rajkumar 2003; Boral et al. 2004; Chauhan and Goyal 2006).

Techniques such as ELISA are widely advocated by researchers around the world for supporting the results of SPT (Rajkumar 2003; Wan-Ishlah and Gendeh 2005; Fereidouni et al. 2009). It is therefore

recommended to use both the tests to gain a definitive diagnosis, as not all sensitivities could be identified with the use of SPT alone. SPT is more sensitive but less specific than serum IgE tests to diagnose allergenicity against potent antigens. Although total IgE is the original screening test for allergy, it is not 100 % specific as total IgE may be elevated due to parasitic infections or family history of atopy. Thus, ELISA is preferred as the specificity of the test is as high as 90 % for the inhaled allergens and is not influenced by concurrent drug treatment.

Heterogeneity was recorded in the binding of specific IgE antibodies of individual patient's sera to different antigenic extracts. This could be due to variation in the sensitivity of patients against the antigens (Peltre et al. 1982; Cookson 1999). The concordance between positive skin reactions and serum-specific IgE antibodies varied for different antigenic extracts. Antigenic extracts of *Artemisia scoparia*, *C. fistula*, *R. communis* and *Z. mays* showed

Table 4 Percent correlation between SPT and ELISA among individual pollen antigens

Pollen antigen	No. of SPT +ve sera tested for ELISA	No. of ELISA +ve sera (percent binding >15 %)	Correlation (%) between SPT and ELISA
<i>Amaranthus spinosus</i>	21	8	42.8
<i>Artemisia scoparia</i>	12	5	66.6
<i>Azadirachta indica</i>	14	7	46.6
<i>Cannabis sativa</i>	17	4	23.5
<i>Cassia fistula</i>	13	9	69.2
<i>Cassia occidentalis</i>	22	5	22.7
<i>Cassia siamea</i>	20	3	15
<i>Cenchrus ciliaria</i>	11	2	18.1
<i>Chenopodium album</i>	15	4	26.6
<i>Chenopodium murale</i>	19	5	26.3
<i>Cynodon dactylon</i>	22	4	18.1
<i>Cyperus rotundus</i>	04	3	20
<i>Eucalyptus tereticornis</i>	11	3	27.2
<i>Impertea cylindrica</i>	13	5	38.4
<i>Morus alba</i>	14	4	35.7
<i>Parthenium hysterophorus</i>	12	4	33.3
<i>Pennisetum typhoides</i>	23	4	17.3
<i>Prosopis juliflora</i>	24	5	25
<i>Ricinus communis</i>	21	13	61.9
<i>Sorghum vulgare</i>	13	7	46.1
<i>Xanthium strumarium</i>	17	8	47
<i>Zea mays</i>	27	14	51.8

more than 50 % correlation between in vitro and in vivo diagnostic tests. Similar to our results, Rawat et al. (2004) also observed 50 % correlation between skin test and in vitro test. Least concordance was

observed against pollen extracts of *C. siamea*, *P. typhoides*, *C. ciliaria*, *C. dactylon* and *C. rotundus*. Sharma et al. (2009) reported 60 % correlation between the in vivo and in vitro tests for antigenic extract of *P. juliflora*; however, in this study, concordance observed was 25 %. In the present study, a moderate concordance was observed between SPT and ELISA, and it is speculated that SPT might give false positive results in some cases. Therefore, an in vitro diagnostic test should be used to confirm the incidence of allergenicity in such cases.

5 Conclusions

The present work is a pioneer attempt to assess the allergenicity of some of the pollen types prevalent in the atmosphere of Rohtak city. Grasses such as *C. dactylon* and *C. ciliaria* and some weeds such as *R. communis* and *Parthenium hyterophorus* are important aeroallergens of the city. As these are wide spread and most potential source of allergy, their management may help in controlling the planned developmental programmes for incidence of allergy. Trees such as *P. juliflora*, *C. occidentalis* and *Morus alba* are also important source of pollen allergens; therefore, urban plantation programmes should take into consideration this fact. In general, it was observed that the dominance of a particular pollen type in the air does not influence its allergenicity.

An important limitation of our work is that the allergenicity of all the pollen types present in the atmosphere of the study area was not tested. Nevertheless, there is a great scope for these results to be used by public health authorities in the prediction of potential pollen types as sources of respiratory allergy in susceptible individuals. Since we have not yet been able to find any study conducted on this subject in this region, we recommend that further comprehensive research is needed to understand the relationship between patients with respiratory allergies and air-borne pollen and to guide the patients on the subject.

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