

Aerial pollen diversity in Punjab and their clinical significance in allergic diseases

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Abstract The present study has been carried out to assess the allergenicity of dominant pollen types from the atmosphere of nine districts of the state of Punjab, India. During the study period (2012–2014), 30 different kinds of pollens belonging to 17 families were recorded. Antigenic extract of thirteen pollen types were selected for skin prick tests (SPT). The allergenic pollens selected for the present study belong to families: Asteraceae, Amaranthaceae, Brassicaceae, Cannabaceae, Chenopodiaceae, Meliaceae, Moraceae, Myrtaceae and Xanthorrhoeaceae. Total 165 sensitized patients having diagnosed with bronchoprovocation test were selected for SPT. Diagnostic tests were performed in ENT department, Rajindra hospital Patiala, and SPT were done in the clinic of Dr. H.S. Bedi at Patiala. Antigenic reactivity (3+ to above) to various allergens varied from 2.4 to 9.09 %. Patients showed maximum allergenic sensitivity to *Parthenium hystrophorus* (9.09 %) followed by *Morus alba* (8.48 %), *Amaranthus spinosus* (7.87 %) and *Ageratum* sp. (6.06 %). Least reactivity was shown by *Brassica campestris* and *Eucalyptus* sp. (2.4 %).

Keywords Pollen · Allergens · Allergenicity · Skin prick test

1 Introduction

Aerobiology is a science dealing with airborne particles of biological origin, carried out and transported passively with the air current. Airborne particles are a major cause of respiratory ailments of humans causing allergies, asthma, and pathogenic infections of the respiratory tract (Essien and Agwu 2013). Allergy is an altered and accelerated reaction of a person to a second or subsequent exposure to substances to which a person has been sensitized during the first exposure which could results to a condition in which the body produces an abnormal immune response to certain antigens which include dust, pollen, certain food and drugs, or fur (Singh and Singh 1994).

Airborne pollen grains of various plants species are responsible for different types of allergies. The pollen grains and fragments transported by the air generally range between 10–50 µm in size. Allergic pollen grains belong to three broad categories of plants: grasses, weeds and trees. Allergy to pollen grains is called pollinosis (Essien et al. 2013; Essien 2014). Generally pollen that causes allergies is that of anemophilous plants. Such plants produced large quantities of light-weight pollen with psilate (pollen wall surface with completely smooth sculpturing)

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exine which can be carried away to great distances and are easily inhaled, bringing them in contact with the sensitive nasal passages (Singh and Rawat 2000). A characteristic feature of pollen allergy is its seasonal occurrence associated with the prevalence of pollen of these particular species in the atmosphere. These airborne pollen grains and spores widely cause various allergic complaints such as hay fever, eczema and in severe cases asthma (Burge and Rogers 2008). In hay fever and asthma responses, pollen grains enter the nose and lands on the mucous membrane of the upper and/or lower respiratory tract. Pollen grains get hydrated by the mucus secretion and release pollen allergens which penetrate the mucous tissues (Foster and Clark 2001). Pollen causing allergy are quite variable in different ecozones which makes it very important to identify pollinosis causing species from every region and prepare extracts from them for diagnosis and immunotherapy for the benefit of allergy sufferers (Singh and Kumar 2004).

In India, the work on pollen allergy was initiated in 1950s by Shivpuri in Delhi and later a great deal of work has been carried out at different eco-geographical regions in India with regard to allergenic pollen flora (Kasliwal and Solomon 1958; Lakhanpal and Nair 1958; Kasliwal et al. 1959; Shivpuri and Parkash 1967; Singh and Shivpuri 1971; Mittre and Khandelwal 1973; Shivpuri et al. 1979; Singh and Babu 1980; Gupta et al. 1984; Malik et al. 1991; Karmakar et al. 1994; Rawat et al. 2004; Sharma et al. 2009; Wani et al. 2010). The dominant pollen types recorded by these scientists are grasses, *Chenopodium*, *Amaranthus*, *Ailanthus*, *Ricinus*, *Morus*, *Xanthium*, *Cannabis*, *Artemisia* and *Holoptelea*. Shivpuri and Parkash (1967) observed *Prosopis juliflora* as a major cause of pollinosis with 12 % patients showing a positive skin reaction. Shivpuri et al. (1979) and Singh et al. (1987) recorded positive skin reactions in 16.9 % patients to *Pinus roxburghii* from the foot hills of Himalayas. Later, important pollens causing allergy from Delhi were identified as *Ageratum*, *Ailanthus*, *Amaranthus*, *Anogeissus pendula*, *Artemisia*, *Cassia siamea*, *Cenchrus*, *Chenopodium*, *Cynodon*, *Ipomoea fistulosa*, *Paspalum distichum* and *Poa annua* (Shivpuri et al. 1979; Singh et al. 1987). From Northern India, *P. juliflora*, *Ricinus communis*, *Morus*, *Mallostus*, *Alnus*, *Quercus*, *Cedrus*, *Argemone*, *Amaranthus*, *Chenopodium*, *Holoptelea* and grasses have been reported as major source of pollen allergens

(Anonymous 2000). Recently under AICP (All India Coordinated Project) on aeroallergens and human health, 43 types of pollen were recorded. The dominant types were *Holoptelea*, Poaceae, *Eucalyptus*, *Casuarina*, *Putranjiva*, etc. According to the study, *Holoptelea* contributed 22.2 % pollen to the air from March to May (Anonymous 2000).

However, no such data are available from the state of Punjab, as it has been not explored for pollen allergens. The objective of this study was to determine the airborne pollen spectrum in the atmosphere of Punjab and allergic reactions caused by them to the sensitive patients.

2 Materials and method

2.1 Study area

The present investigation was carried out in state of Punjab, located at the latitude 30°4' North and longitude 75°5' East with an area of 50,362 km² in northwestern India. Nine districts were selected for the present study, viz. Amritsar, Bhatinda, Faridkot, Gurdaspur, Hoshiarpur, Ludhiana, SBS Nagar, Patiala and Roopnagar (Fig. 1). Atmospheric survey of airborne pollens was carried out for two consecutive years (August 2012–July 2014). The year was divided into 3 seasons: summer season (March–June), rainy season (July–Oct) and winter season (Nov–Feb). Collection was done twice in the year from the collection site. Proper sites for the collection of pollens were chosen. The pollens were collected using a personal volumetric glass slide sampler (Gautam Enterprises, New Delhi). It is portable, battery-/power-operated sampler with a flow rate of 10 L/min. It has a rectangular orifice at top for suction of air and a slit on the side to insert the glycerin-coated microslide. Samples were collected from the sites at three times a day (morning, afternoon and evening) for practical convenience. A drop of glycerin was added to each exposed slide before covering it with a 22 × 22 glass cover slip. The slides were studied under Leica microscope at 10×, 40× and oil emersion (100×) magnification. Ringing of slides was done with the help of nail polish. The pollens were identified with the reference of known taxa and also the literature given by Erdtman (1952), Lewis et al. (1984) and Nair et al. 1986.

Fig. 1 Map showing localities of pollen collection (source- punjab.gov.in)



3 Clinical investigation

The diagnosis of pollen allergy was based on the clinical history, ascertained by a positive skin prick tests (SPT). Patients were excluded if they had allergic symptoms caused by other allergens during the pollen season. A positive skin prick test associated with clinical disease is considered a fairly reliable evidence of that specific allergen causing the clinical disease.

3.1 Antigen procurement

A total of 13 pollen types causing allergies and prevalent in the airspora of study area were selected

for assessing their level of allergenicity among patients. The sterilized antigenic extracts of *Ageratum* sp., *Asphodelus* sp., *Amaranthus spinosus*, *Brassica campestris*, *Cannabis sativa*, *Chenopodium murale*, *Chenopodium album*, *Imperata cylindrical*, *Eucalyptus* sp., *Morus alba*, *Parthenium hystrophorus*, *Penisetum typhoides* and *Xanthium strumarium* were procured from ALCIT laboratories, New Delhi, India.

3.2 Selection of patients

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 (at least 48 h) and other sympathomimetic drugs (at least 18–12 h) before the test and have not received corticosteroids for last 3 weeks. Prior informed written consent for performing skin prick tests was obtained from all the subjects. Along with the patients, some volunteer normal persons (controls) were also tested to see the sensitization caused by these allergens. Total 400 patients were studied out of which 165 patients were selected for skin prick tests.

3.3 Skin prick tests (SPT)

A total of 165 patients with nasobronchial symptoms attending ENT department, Rajindra Hospital Patiala, during 2012–2014 were selected for skin prick test. The flexor aspects of the forearm or the lateral aspect of upper arm of the patient was used as the site for testing. Skin prick test was performed by a trained paramedic under the supervision of a senior doctor, Dr. R.S. Bedi in his clinic at Patiala. The study was approved by the ethical committee of the concerned institute.

Buffer saline was used as negative control and histamine acid phosphate as a positive control. The significance of negative control is that it shows the physiological conditions and general reactivity of skin, whereas the positive control shows the skin reactivity to minute dose of histamine and to what extent. A 26-gauge tuberculin syringe with $\frac{1}{2}$ inch bevel sterile hypodermic needle was used for injection, and 0.01 ml of the solution was injected intradermally. This raised bleb of 2 mm, which in 15–20 min attained the size of 4–5 mm without an erythema. A separate syringe and needle were used for each antigen. A distance of 4 mm was kept between two skin prick test sites. The reaction was examined for 1 h at an interval of 15–20 min. The strength of each reaction by the degree of erythema and area of weal formed was observed and compared with the controls.

3.4 Inclusion/exclusion criteria

Patients in the age group of 18–54 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 allergic or anaphylaxis and chronic debilitating diseases.

4 Result

During the study period, 30 different kinds of pollens were recorded from different localities (Table 1) belonging to 17 families. A total of 13 different kinds of pollens were characterized for their allergenic properties. The pollens with allergenic properties belong to ten families, viz. Meliaceae, Xanthorrhoeaceae, Amaranthaceae, Brassicaceae, Cannabinaceae, Chenopodiaceae, Myrtaceae, Moraceae, Poaceae and Asteraceae. The most prevalent type of pollens observed during the present study belongs to the family Asteraceae, Moraceae and Myrtaceae.

4.1 Seasonal abundance of the pollens identified under the microscope has been described in Table 1

A total of 400 patients were studied who visited the hospital during the present study period 2012–2014 having susceptible to allergic reactions. Out of 400 patients, 225 (56.25 %) were males and 175 patients (43.8 %) were females belonging to the average age of 36.5 ± 17.4 and 35.4 ± 17.8 , respectively (Table 2). Bronchoprovocation tests were performed on all the patients, and out of 400 patients, 165 patients with allergic respiratory diseases were selected for skin prick test (Table 3). On the basis of skin prick test performed, only 70 patients were found to be sensitive to 13 pollen allergens (Table 4).

Though the reactivity shown by the antigens were almost equal among the male and female patient groups, there was a slight increase in positivity among the male group. The skin reactions were classified as positive, markedly positive and highly positive as summarized in Table 5. Patients showing more than 2+ reactions were considered as markedly positive, and those showing 3+ or above reactions were considered as highly positive. Most of the patients were sensitive to *P. hystrophorus* followed by *M. alba*, *Ageratum* sp. and *A. spinosus* and were least sensitive to *B. compestris* and *Eucalyptus* sp. Antigenic reactivity (3+ to above) varied from 2.4 to 9.09 %. It was found to be maximum for *P. hystrophorus*, i.e.,

Table 1 Seasonal abundance of pollens identified from under the microscope different districts of Punjab

S. no.	Pollen type	Family	Seasonal abundance of pollens		
			March–June	July–Oct	Nov–Feb
1.	<i>Acacia nilotica</i>	Fabaceae	+	–	+
2.	<i>Bauhinia purpurea</i>	Caesalpiniaceae	–	+	–
3.	<i>Cassia fistula</i>	Caesalpiniaceae	+	–	–
4.	<i>Dalbergia sissoo</i>	Papilionaceae	+	–	–
5.	<i>Delonix regia</i>	Leguminosae	+	+	–
6.	<i>Albizia</i> sp.	Fabaceae	+	–	+
7.	<i>Ageratum</i> sp.	Asteraceae	–	+	–
8.	<i>Amaranthus spinosus</i>	Amaranthaceae	+	–	–
9.	<i>Asphodelus</i> sp.	Xanthorrhoeaceae	+	–	–
10.	<i>Azadirachta</i> sp.	Meliaceae	+	–	–
11.	<i>Brassica campestris</i>	Brassicaceae	+	–	–
12.	<i>Cannabis sativa</i>	Cannabaceae	+	+	–
13.	<i>Carica papaya</i>	Cariaceae	–	–	+
14.	<i>Chenopodium album</i>	Chenopodiaceae	+	–	+
15.	<i>Chenopodium murale</i>	Chenopodiaceae	+	–	+
16.	<i>Citrus</i> sp.	Rutaceae	–	+	+
17.	<i>Callistemon viminalis</i>	Myrtaceae	+	–	–
18.	<i>Eucalyptus</i> sp.	Myrtaceae	+	–	+
19.	<i>Eugenia jambolana</i>	Myrtaceae	+	–	+
20.	<i>Mangifera indica</i>	Anacardiaceae	+	–	+
21.	<i>Melia azedarach</i>	Meliaceae	+	–	–
22.	<i>Ficus religiosa</i>	Moraceae	–	–	+
23.	<i>Morus alba</i>	Moraceae	+	–	–
24.	<i>Nerium indicum</i>	Apocynaceae	–	+	–
25.	<i>Ocimum sanctum</i>	Lamiaceae	–	–	+
26.	<i>Parthenium hysterophorus</i>	Asteraceae	–	–	+
27.	<i>Pennisetum typhoides</i>	Poaceae	–	–	+
28.	<i>Imperata cylindrica</i>	poaceae	+	–	–
29.	<i>Xanthium strumarium</i>	Asteraceae	–	+	–
30.	<i>Ziziphus jujuba</i>	Rhamnaceae	+	+	–

Table 2 Descriptive characteristics of patients who visited the allergy clinic

	Males (%)	Females (%)	Total
Number of individuals	225 (56.25 %)	175 (43.8 %)	400
Age of individuals (mean $\pm$ SD)	36.5 $\pm$ 17.4	35.4 $\pm$ 17.8	35.9 $\pm$ 17.6
Positive results for skin prick test	160 patients (96.96 %)		
Negative results for skin prick test	5 patients (3.03 %)		
Total patients on whom skin prick test was conducted	165 (41.25 %)		

(9.09 %) followed by *M. alba* (8.48 %), *Ageratum* sp. (8.48 %) and *A. spinosus* (7.87 %), whereas *B. campestris* (2.4 %) and *Eucalyptus* sp. (2.4 %) showed least antigenic reactivity. Markedly positive skin reactions (2+ to 4+) were shown by the patients that varied from 8.48 to 16.9 %. Maximum antigenic

showed least antigenic reactivity. Markedly positive skin reactions (2+ to 4+) were shown by the patients that varied from 8.48 to 16.9 %. Maximum antigenic

Table 3 Gender and age of individuals on whom skin prick test was conducted

	Males (%)	Females (%)	Total
Number of individuals	90 (54.54 %)	75 (45.45 %)	165
Age of individuals (mean $\pm$ SD)	35.3 $\pm$ 16.5	34.5 $\pm$ 16.8	34.9 $\pm$ 16.5

Table 4 Number, percentage and age of individuals sensitive to pollen allergens

Allergen type	Number	Percentage	Age M/F
Pollen allergens	70	42.4	34.4 $\pm$ 16.7/ 33.6 $\pm$ 15.8

Table 5 Number of individuals sensitized to different types of pollens

S. no.	Pollens	Positive (1+ to 4+)		Markedly positive (2+ to 4+)		Highly % positive (3+ and above)		Total number of individuals	Percentage (%) (total)
		No.	(%)	No.	(%)	No.	(%)		
1.	<i>Ageratum</i> sp.	21	14.5	22	13.9	14	8.48	57	34.55
2.	<i>Asphodelus</i> sp.	17	10.3	15	9.09	5	3.03	37	22.42
3.	<i>Amaranthus spinosus</i>	20	13.3	18	11.5	13	7.87	51	30.91
4.	<i>B. compestris</i>	17	10.3	14	8.48	4	2.4	35	21.21
5.	<i>Cannabis sativa</i>	23	13.9	20	12.1	9	5.4	52	31.52
6.	<i>Chenopodium murale</i>	21	12.7	20	12.1	5	3.03	46	27.88
7.	<i>Chenopodium album</i>	23	13.9	21	12.7	5	3.03	49	29.69
8.	<i>Eucalyptus</i> sp.	15	9.09	17	10.3	4	2.4	36	21.82
9.	<i>Imperata cylindrica</i>	20	12.1	21	12.7	6	3.63	47	28.48
10.	<i>Morus alba</i>	21	12.7	23	13.9	14	8.48	58	35.15
11.	<i>P. hystrophorus</i>	26	15.7	28	16.9	15	9.09	69	41.82
12.	<i>Pennisetum typhoides</i>	20	12.1	23	13.9	9	5.4	52	31.52
13.	<i>Xanthium strumarium</i>	21	12.7	22	13.3	8	4.84	51	30.91

reactivity was shown by *P. hystrophorus* (16.9 %), followed by *M. alba* (13.9 %), *P. typhoides* (13.9 %) (written two times and has been corrected), *X. strumarium* (13.9 %), *Imperata cylindrica* (12.7 %) and *C. album* (12.7 %), whereas *B. compestris* (8.48 %) showed least antigenic reactivity.

5 Discussion

The pollen allergy depends on the combined effects of several factors: the patient, the allergens, the timing, the duration of exposure and the qualities of the environment. Studying plants pollen levels in relation to meteorological elements is of high practical importance because of its health concern

(Makra et al. 2006). Pollen causing allergy is quite variable in different ecozones, which makes it very important to identify pollinosis causing species from every region and prepare extracts from them for diagnosis and immunotherapy for the benefit of allergy sufferers.

Therefore, an attempt was made to identify the pollen allergens of various districts of state of Punjab. 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 allergens ailments (Singh et al. 1987; Wan-Ishlah and Gendeh 2005; Mandal et al. 2008; Fereidouni et al. 2009; Wani et al. 2010; Ahlawat et al. 2014).

The percentage of patients showing markedly positive skin reactions to antigenic extracts of 13 pollens varied from 2.4 to 16.9 % with an average of 9.65 %. The overall incidence of SPT reactivity was highest against the antigenic extract of pollen belonging to family Asteraceae and Moraceae. High prevalence of grass pollen allergy has been 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; Ahlawat et al. 2013). Dua and Shivpuri (1962), Shivpuri et al. (1979), Singh et al. (1987), Rajkumar (2003) have reported *Cynodon* sp., *Imperata* sp. and *Pennisetum* sp. as the most common aeroallergens from Delhi, and this is in accordance with the present study in which *imperata* sp. and *pannisetum* sp. have been found to be the most common aeroallergen whereas *cynodon* spp was altogether absent.

Chenopodium murale, *C. album*, *Imperata cylindrica*, *A. spinosus* and *Xanthium strumarium* were also among important allergens eliciting skin reactivity in 27.88, 29.69, 28.48, 30.91 and 30.91 % of the subjects, respectively, and have been observed during the present study. *A. spinosus* has been shown to be predominant allergen from Delhi (Singh and Dahiya 2002). Sharma et al. (2009) also reported high positivity (23.5 %) against the antigenic extract of this pollen from Assam. In our study, low incidence of positivity was found against the antigenic extract of *Eucalyptus* sp. and *B. campestris*. Our findings are in accordance with the findings of Agnihotri and Singh (1971) and Prasad et al. (2009). According to them, *Eucalyptus* sp. did not show any markedly positive skin reaction among the patients of nasobronchial allergy. In our study, the antigenic extract of *P. hystrophorus*, *M. alba* and *Ageratum* sp. showed high incidence of allergenicity, i.e., 41.82, 35.15 and 34.55 %, respectively. These findings are in accordance with Tilak and Vishwanathan (1980), Agashe et al. (1983), Malik et al. (1990), Shukla and Shukla (2010), Subbarao et al. (1985), Singh and Kumar (2003), Mandal et al. (2006), whereas these findings are in contrary to the observations made by Rajkumar (2003), Boral et al. (2004), Chauhan and Goyal (2006) from other parts of the country where they registered moderate skin reactivity to *P. hystrophorus* and *M. alba*. Singh and Kumar (2004) reported the maximum skin reactivity against *Rumex acetosa* and *Ailanthus excelsa* (17.6 %), followed by *Trewia nudiflora*

(9.7 %), *Argemone mexicana* (9.5 %) from Chandigarh, and in Delhi 12.6 % of the atopic population were found to be positive against *A. spinosus*, 8.46 % to *Populus deltoids* and 7.46 % against *Dodonea viscosa* and *Bauhinia vareigata*.

The role of the different pollen allergens varies with environment conditions, such as climatic factors, pollution and degree of exposure. The knowledge on diurnal, seasonal and annual fluctuations in airborne pollen in any geographical area is essential for effective diagnosis and treatment of pollen allergy. Because of change in the climatic conditions, observation on diurnal and seasonal prevalence becomes very important (D'Amato et al. 2002). Therefore, a continuous monitoring of aerial pollen diversity is important.

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