Allergy Testing – An Overview

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Childhood allergies pose huge economic burden and adverse effects on quality of life. Serum IgE has been considered a surrogate allergy marker for decades. Availability of several over-the-counter allergy tests add to confusion of partially trained caregivers. The present review focuses on current status of allergy testing in Indian scenario. Various *in-vitro* and *in-vivo* diagnostic modalities are available for allergy detection. Skin prick tests are useful for aero-allergies whereas oral challenge tests are best for identifying suspected food allergies. An allergy test should be individualized based on clinical features, diagnostic efficacy, and cost-benefit analysis.

Keywords: *Challenge test, Histamine, Hypersensitivity, IgE, Patch test, Skin prick test*.

Allergy encompasses a wide spectrum of manifestations affecting skin, respiratory and gastrointestinal systems. Several genetic, environmental and socio-economic factors play an important role in the diverse presentation. A rising trend of allergies has been noted worldwide affecting physical, social and psychological well-being and increasing economic burden and disability adjusted life years [1,2]. It is estimated that about 20-30% of the Indian population suffers from atleast one form of allergy, of which, rhinitis is most common followed by asthma [3,4]. Scarce diagnostic facilities and limited knowledge further add onto the disease burden. This review focuses on various available modalities for allergy diagnosis and their clinical relevance.

TERMINOLOGY

As per World Allergy Organization (WAO) and European Academy of Allergology and Clinical Immunology (EAACI), hypersensitivity is defined as a state when there are objectively reproducible symptoms or signs initiated by an exposure to a defined stimulus at a dose tolerated by other persons [5]. Allergy is a chronic clinical condition involving an abnormal immune reaction to an ordinarily harmless allergen, commonly mediated by IgE production though other mechanisms can play significant role [6]. An IgE-mediated allergy with personal or familial tendency is 'atopy'. Hyper-sensitivity reactions usually have an immunological basis but all are not allergies. This understanding is necessary as most of the available allergy tests can only detect hypersensitivity in response to a particular object/allergen, which requires clinical correlation to be labelled as allergy.

Types of Hypersensitivity Reactions

1. *Type I (Immediate or IgE-mediated)* – Rapid immunologic reaction in a previously sensitized individual, triggered by binding of an antigen to IgE antibodies on the surface of mast cells [7].
2. *Type II (Antibody-mediated cytotoxic/cytolytic)* – IgG- and IgM- mediated cellular damage with complement and phagocyte involvement.
3. *Type III (Immune complex mediated)* – Antigen-antibody immune complex deposition causing complement activation and tissue damage.
4. *Type IV (Cell-mediated or delayed hypersensitivity)* – Direct cell damage mediated by various cytokines released by sensitized Th1 cells.

Most of the clinically relevant allergies are mediated *via* type I hypersensitivity. Commonly used *in vitro* tests detect free IgE, whereas bound IgE and mast cell degranulation can be demonstrated by *in vivo* procedures (skin-prick or challenge tests). Food allergies have various IgE (*e.g.*, urticaria, angioedema, asthma, rhinitis, anaphylaxis and oral allergy) and non-IgE (*e.g.*, dermatitis hereptiformis, Heiner's syndrome, procto-enterocolitis, enteropathy and Celiac disease) mediated mechanisms; hence, IgE-based tests alone are insufficient for their diagnosis [8]. Atopic dermatitis and eosinophilic disorders may have both types of mechanisms.

ALLERGY DIAGNOSIS

The key lies in detailed clinical history, relevant physical examination and knowledge about local environment. Temporal relationship of allergen exposure and onset of clinical features, periodicity of symptoms (seasonal/ perennial, diurnal), aggravating/relieving factors, history of travel, pet or insect exposure, number of affected body systems, familial atopy and occupational history should be elicited. ***Table* I** enlists common indoor allergens and associated risk factors. Attending physician/allergist should be aware of local aeroallergens with seasonal variation (***Web Table* I**) and regional predominance (***Web Table* II**). Knowledge about regional pollen calendar combined with clinical correlation can help in tailoring allergen panel for individual patient.

Laboratory Tests

Tests should be carefully selected based on patient history, environmental triggers and operational issues (cost, anaphylaxis risk, time required). Tests may be targeted towards either cause identification or functional and structural disability assessment.

Immunological Tests: For Cause Identification

In vitro tests

(*i*) *Total IgE levels* – A reaginic antibody was discovered by Ishizaka (1966) and Johansson (1967) groups independently and named as IgE by WHO in 1968 [6]. Serum total IgE levels, a conglomerate of all specific IgE molecules, are neither sensitive nor specific for allergy diagnosis [9]. Raised levels may be documented in conditions like parasitic infestations, immunodeficiency disorders (*e.g.*, AIDS, hyper IgE syndromes etc.), Ebstein Barr virus (EBV) infection, rheumatoid arthritis and smoking [10]. IgE molecules produced against specific individual antigens are labelled as serum specific IgE (sIgE). These were detected by Radio-Allergo-Sorbent Test (RAST) using radiolabeled (I^{125}) antihuman IgE molecules. Radio-isotopes have now been replaced by enzyme conjugated antihuman IgE antibodies (Immunocap). The major pitfall of sIgE estimation is false positivity with high total IgE levels (>300 kIU/L) due to non-specific binding to test allergens [11].

(*ii*) *Component resolved diagnostics (CRD)*: Epitopes on some allergens may have structural homology with others. For example, allergenic epitopes on birch pollen share similar structural characteristics to peanut and hazel nut (***Web Table* III**), which may be responsible for oral pruritus while eating fresh nuts in a birch pollen allergic individual without any true nut allergy (oral allergy syndrome). During testing with crude allergen extract for either of them there could be false positive reaction to others due to phenomenon of cross reactivity. To combat this problem, recombinant allergens using a specific epitope are being manufactured to improve diagnostic efficacy of in-vitro food allergen tests [12] and better management of the patients [13]. The high-risk component allergenic proteins for peanut (Ara h1, 2, 3, 9), hazelnut (Cor a8, 9, 14), walnut (Jug r1, 2, 3), soya (Gly m5, 6), wheat (Tri a14, 19) and Rosacea fruits (Pru p3, Mal d3) can be detected with CRD [13]. It is a promising tool for improving the specificity in allergy testing though more studies are required for its validation.

In vivo tests

(*i*) *Skin prick test (SPT)* – SPT is an age-old technique first described by Charles Harrison Blackley (1860s) in patients of 'hay fever'. It detects bound IgE by replicating a mini allergic reaction in the already sensitized host once the allergens are delivered epicutaneously. SPT is considered "gold standard" test for diagnosing IgE mediated allergic diseases [14].

SPT should be performed at a place well equipped with resuscitation facilities. As, blood vessels and pain receptors are located in deep dermis, SPT is pain-free and associated with minimal risk of bleeding or infection if

TABLE I COMMON INDOOR ALLERGENS

Organism	*Allergen*	*Location*	*Risk factors*
Dust Mite	Der p1 and Der f1	Carpet, bedding (mattress/pillow/ curtains) and upholstery	Humidity, older homes and absence of air-conditioning
Dog	Can f1 and albumin	Carpet and bedding; airborne	Depends on the breed and/or animal
Cat	Fel d1	Carpet and bedding; airborne	Cat allergen is universal
Cockroach	Bla g1-4 and Per a1	Kitchen and dining	Humidity and open food sources
Fungus	Various	Bathrooms and/or kitchens; areas of water damage	Humidity, water damage, and leaky plumbing

Der p: Dermatophagoides pteronyssinus; Der f: Dermatophagoides farinae; Can f: Canis familiaris; Fel d: Felis domesticus; Bla g: Blattella germanica; Per a: Periplaneta americana.

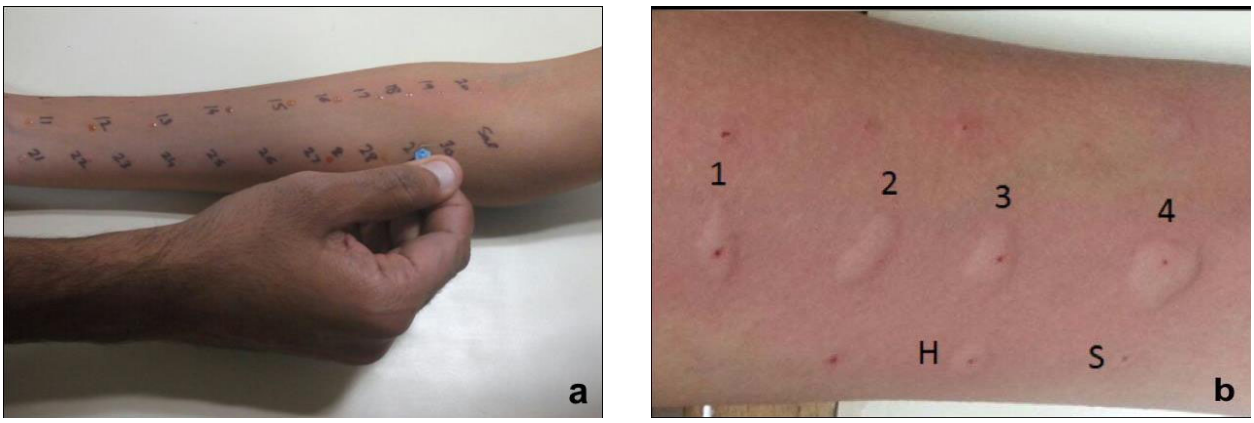

FIG. 1 *Skin prick test: (a) technique; (b) 'Wheal' and 'Flare' reactions after SPT to different allergens (1,2,3,4).*

performed appropriately (***Fig.* 1a**) [15]. Crude extract may directly be pricked (Prick test) followed by SPT, in case of non-availability of standard allergen extract [16]. Selection of antigens should be based upon patient's clinical and environmental history, occupation and socio-economic factors. House dust, house dust mite, relevant pollens (grass, tree or weeds), fungus (Alternaria, Aspergillus), insects (Cockroach) and pet animals (dog, cat, buffalo) dander are the commonest aeroallergens prevalent in India whereas milk, egg, peanut, soya, wheat, tree nut, fish and shell fish contribute to majority of food allergens [17,18]. Cockroach remains the predominant allergen among insects whereas common occupational allergens are latex and chemicals. Honey bees are important allergens in high risk groups like bee keepers. A patient with either recurrent or persistent symptoms not adequately controlled by preventer therapy should be tested with SPT. Patients with allergic rhinitis or asthma having either persistent or moderate to severe symptoms as per the ARIA and GINA guidelines should be subjected to allergy testing. Patients on anti-histaminics and immunomodulators, on b-blockers, with unhealthy skin condition, within 4 weeks of anaphylaxis and extremes of age are not suitable for SPT. Clinical correlation of test results might help in instituting specific allergen avoidance measures and targeted immunotherapy in select cases.

Histamine and normal saline serve as positive and negative controls respectively and read after 10 minutes and 15 minutes [16]. Positive reaction is suggested by appearance of a wheal at the prick site (***Fig.* 1b**). The maximum diameter of the wheal is measured and reaction interpreted in millimeters (mm) of wheal diameter [19, 20]. Also, any pseudopod (an extra protuberance to the regular circular shape) if observed, is separately mentioned. Positive control should be at least 3 mm or more than negative control to establish test validity. Any reaction with normal saline more than 3 mm should be considered as baseline high reactivity of the skin, making test conditions invalid. If positive and negative controls are within 3 mm of each other, the test should be considered invalid. Any allergen showing a wheal size of ≥3 mm than the negative control should be considered positive indicator of hypersensitivity. Any wheal size more than 8 mm suggests high positive predictive value.

Factors influencing SPT

- *Medications* – Certain medications may affect the results of SPT as mentioned in ***Table* II** [16]. SPT reaction usually declines after 6 months to 3 years of allergen immunotherapy.
- *Age* – SPT is currently practiced beyond 6 months of age though no lower or upper age limit cutoff is recommended [16]. Skin reactivity declines after 60 years.
- *Test area* – The mid and upper back are 33% more reactive than the lower back. The back as a whole is more reactive (53%) than the forearm. An area approximately 5 cm away from the wrist and 3 cm from the antecubital fossa, on the forearm is usually used [16]. Left forearm is generally preferred.

TABLE II DRUGS TO BE STOPPED BEFORE SKIN PRICK TEST

Medication	*Period for withholding before test*
Antihistaminics (H_1 blockers)	48 hours
Astemizole	60 days
Ketotifen	5 days
Tricyclic antidepressants	2 weeks
Short-term (topical/systemic) steroids	No effects
Long-term systemic steroids	2 weeks
Long-term topical steroids	2-3 weeks

- *Distance between two pricks* – A minimum of 2 cm gap should be present between two adjacent test sites. This is to prevent non-specific enhancement through nearby axon reflex and also to avoid merging of wheals from strong reactions in the nearby area.
- *False positives* – Skin conditions (dermatographism, acute or chronic urticaria, cutaneous mastocytosis), naturally occurring histamine in some allergen extracts (insect venom, mold, foods), non-standard allergen preparations (irritant reaction), cross-reactivity with homologous proteins.
- *False negatives* – Recent (within 4 weeks) anaphylaxis, medications (***Table* II**), technical (low-potency extract, false technique), UV exposure.

The advantages of *in vitro* tests include no effect of anti-histaminics or steroids, feasibility with any skin condition and no risk of systemic reactions. Serum sIgE has better specificity with higher positive predictive value for determining aeroallergen (pollen and insects) sensitization [21]. SPT have an edge over in-vitro tests in terms of better sensitivity with clinical correlation, faster result (15-20 minutes), no interference with high IgE levels and cost effectiveness [16]. Intradermal tests, with more risk potential, are indicated only when SPT and/or sIgE results are negative with relevant exposure history [22].

(ii) *Patch test* – It is based on delayed (type IV) hypersensitivity. Patches with allergenic proteins are applied on the upper back. An eczematous reaction usually occurs after 48-72 hours till as late as 7 days. Optimum reading time is day 2, 4 and 7 after patch application [23]. Test reactions are graded as erythema, vesiculation or ulceration. TRUE test is one of the commercially available patches with approximately 30 allergens. The most common allergens are nickel sulfate, neomycin, myroxylon pereirae (balsam of Peru), fragrance mix, thiomersal, sodium gold thiosulfate, quaternium-15, formaldehyde, bacitracin and cobalt chloride [24]. Patch test can be used at any age with negligible risk of anaphylaxis. It has high specificity with very low sensitivity and is time consuming.

(iii) *Nasal provocation test* – Increasing quantities of allergen extract are introduced in the anterior part of inferior nasal turbinate to reciprocate the allergic reaction. Due to higher chances of anaphylaxis and cumbersome technique, this is not recommended.

(iv) *Bronchial (Methacholine) challenge test* – Bronchoconstriction provoked by methacholine can be quantified by spirometry. It should be done only in hospital setting with availability of emergency facilities and is rarely performed now-a-days.

(v) *Oral food challenge test* – Double blind placebo control food challenge (DBPCFC) test is the gold standard technique for detecting sensitivity to suspected food items. However, due to practical difficulty in masking of food in a vehicle each time, open label food challenge serves the needful [25]. When sIgE or SPT have diminished substantially during the course of food allergy or in case of false positive or negative skin or blood tests, oral challenge can be used to confirm or rule out allergy.

(vi) *Elimination trial test* – In case of true allergy the symptoms should disappear with food elimination and reappear with its reintroduction [26].

Functional Assessment

Airway Hypersensitivity

Spirometry – Though gold standard for functional assessment, effort dependence and requirement of patient cooperation limits its practical utility in younger children and elderly. Evidence of reversible bronchoconstriction is considered consistent with diagnosis of asthma.

Impulse oscillometry – Determines airway resistance, reactance and impedance using sound waves [27]. It can detect peripheral airway obstruction, with minimal patient's cooperation, which may be missed by conventional spirometry [28].

Peak expiratory flow rate – Role limited to monitor lung functions during domiciliary care. Diurnal variability in lung functions is consistent with diagnosis of asthma.

Nasal Patency Assessment for Nasal Allergy

Rhino-manometry detects variable resistance encountered to airstream in nasal passage. Peak nasal inspiratory flowmeter is an economical, fast, portable, easy to use objective test with good reproducibility.

Structural Assessment

Nasal endoscopy: It provides accurate assessment of disease and anatomical variation in patients with symptoms of rhinitis which may be important during surgical interventions. Apart from revealing classical signs (watery nasal discharge and edematous mucosa) it can help in detecting structural deformities (deviated septum, septal spur, polyps, turbinate hypertrophy and stenotic or accessary maxillary ostia), ruling out foreign bodies and collecting samples (for cyto-, microbio- and histopatho-logical examination).

Gastrointestinal (GI) endoscopy: It may be helpful during diagnosis (esophageal edema, furrows, exudates, transient rings and diffuse narrowing) and management

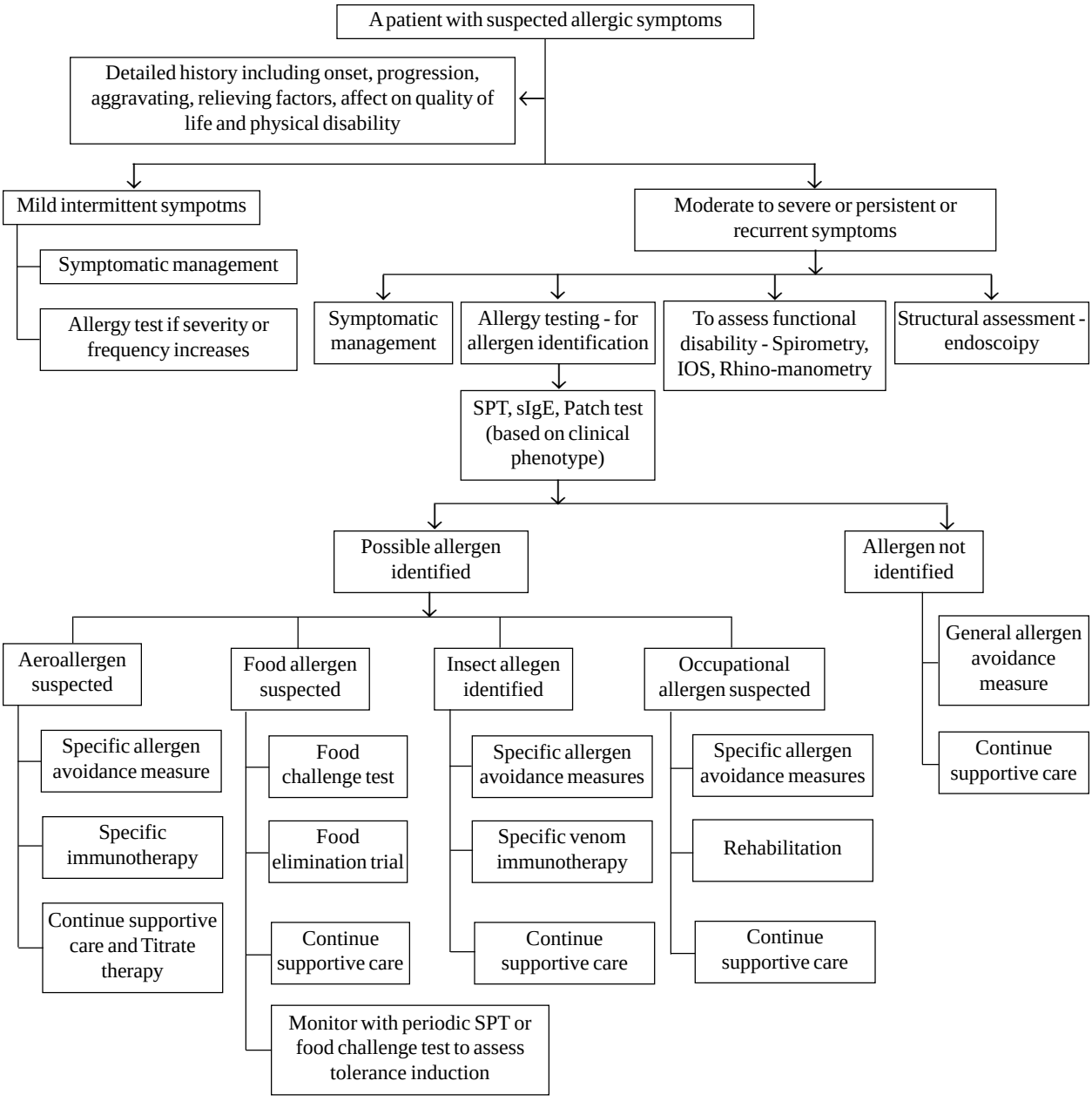

Fig. 2 *Approach to an allergic patient.*

(stricture dilatation) in eosinophilic esophagitis (EoE). Endoscopic guided GI tract biopsy may provide a vital clue for eosinophilic inflammation.

Supportive Tests

Blood eosinophil levels: Hyper-eosinophilia (>450 cells/mL) may be present in Hodgkin lymphoma, Addison disease, allergies (including asthma, eczema, allergic bronchopulmonary aspergillosis, EoE), collagen vascular disorders, drug reactions, mastocytosis, hypereosinophilic syndrome (≥1500 eosinophils/mL) and infections (HIV, parasitic and fungal infections).

Nasal and sputum eosinophilia: It has been documented in patients with allergic asthma.

Fractional Exhaled Nitric Oxide (FENO): FENO is a good surrogate marker for eosinophilic airway inflammation and can assist in treatment of refractory asthma cases.

Mast cell mediators: High histamine levels soon after

KEY MESSAGES

- Blood tests target free IgE while skin tests and challenge tests mimic natural reactions by targeting bound IgE and mast cell degranulation.
- Skin prick test is considered the gold standard for aero-allergen identification, while challenge tests take precedence in suspected food allergies.
- Allergy testing is recommended in moderate-severe or persistent or recurrent symptoms.
- Clinical symptoms, local flora and occupational exposure should be kept in mind while selecting an allergy panel.

anaphylaxis is the best investigation but practically impossible due to short half-life (2-3 minutes). Serum tryptase levels peaks between 15 to 20 minutes (half-life of 2.5 hours.) Blood samples are collected at 1, 2, 3, 6, 12 and 24-hours post-reaction to document both rise and fall. A peak concentration of >50 mg/L with relevant symptoms and documented fall during convalescence is consistent with IgE-mediated anaphylaxis [29].

PITFALLS OF ALLERGY TESTING

The main limitation of allergy testing is that it detects only IgE-mediated hypersensitivity state, which may not be clinically relevant. sIgE may be falsely positive with high total IgE levels. SPT might be falsely negative after certain medications and anaphylaxis.

CONCLUSION

Serum and skin tests help in detecting IgE-mediated hypersensitivities, which require clinical correlation. While choosing an allergy test panel, one needs to be vigilant about relevant allergens as per exposure history and cost to benefit ratio for an individual patient. SPT is a reliable, cost and time effective modality when performed using standard extracts. Patch test may be useful in delayed hypersensitivity reactions. Component resolved diagnostics, a futuristic tool, might be helpful in cross reactive food allergies. 'Less is more' should be the dictum, regarding number of allergens to be tested. Identification of responsible allergens helps in specific allergen avoidance measures and targeted immunotherapy. ***Fig.* 2** gives an algorithmic approach to an allergic patient.

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WEB TABLE I SEASONAL VARIATION OF AEROALLERGENS IN INDIA

Seasons	*Tree Pollens*	*Grass Pollens*	*Weed Pollens*
Spring (Feb-April)	Ailanthus, Holoptelea, Casuarina, Prosopis, Mallotus, Putranjiva, Bauhinia, Quercus	Cynodon, Dicanthium, Imperata, Polypogon, Paspalum, Poa	Cannabis, Chenopodium, Parthenium, Suaeda, Plantago
Autumn (Sept-Oct)	Anogeissus, Eucalyptus, Cedrus, Cocos, Prosopis, Mallotus, Phoenix, Quercus	Bothriochloa, Cenchrus, Hetropogan, Pennisetum, Sorghum	Amaranthus, Artemisia, Cassia, Ricinus, Xanthium
Winter (Nov-Jan)	Cassia, Cedrus, Mallotus, Salvadora, Quercus	Cynodon, Eragrostis, Poa, Phalaris	Ageratum, Argemone, Chenopodium, Asphodelous, Ricinus

WEB TABLE II RELEVANT AEROALLERGENS IN DIFFERENT REGIONS OF INDIA

	North	*South*	*East*	*West*
Tree pollen	Ailanthus, Alnus, Azadirachta, Cassia, Cedrus	Cassia, Cocos	Alnus, Casuarina, Cedrus, Cocos	Ailanthus, Borassus
Weed pollen	Argemone, Artemisia,Brassica, Chenopodium	Artemisia	Artemisia, Brassica, Chenopodium	Artemisia, Brassica
Fungal	Alternaria, Aspergilli, Candida, Cladosrorium, Curvularia, Helminthosporium, Mucor, Nigrospora, Phoma, Smuts	Ascospores, Aspergilli, Candida, Cladosporium, Curvularia, Helminthosporium, Mucor, Smuts, Uredospores	Alternaria, Candida, Cladosporium, Curvularia, Phoma	Alternaria, Aspergilli, Candida, Cladosporium, Curvularia, Helminthosporium, Mucor, Nigrospora, Smuts

Northern India: Jammu and Kashmir, Himachal Pradesh, Haryana, Punjab, Rajasthan, Delhi and Union Territory of Chandigarh; Western India: Gujarat, Maharashtra and Goa; Southern India: Andhra Pradesh, Karnataka, Kerala, Tamil Nadu and the Union Territory of Puducherry; Eastern India: Bihar, West Bengal, Odisha, Jharkhand, Andaman and Nicobar Islands.

WEB TABLE III CROSS REACTIVE ALLERGENS RESPONSIBLE FOR ORAL ALLERGY SYNDROME

Pollens	*Food*
Birch	Apple, peach, plum, pear, cherry, apricot, almond, celery, carrot, parsley, caraway, fennel, coriander, soybean, peanut, hazelnut
Ragweed	Cantaloupe, honeydew, watermelon, zucchini, cucumber, banana
Mugwort	Celery, carrot, parsley, caraway, fennel, coriander, mustard, cauliflower, cabbage, broccoli, garlic, onion
Orchard	Cantaloupe, honeydew, watermelon, peanut, potato, tomato
Timothy	Swiss chard, orange