Original Articles

WHEAT THRESHING DUST - A "NEW ALLERGEN" IN APRIL -MAY NASOBRONCHIAL ALLERGY

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Objective: *To identify the allergen(s) responsible for mid April - mid May nasobronchial allergy seen in North India.* ***Design:*** *Case control study.* ***Setting:*** *Children living in and around Chandigarh (urban, rural).* ***Subjects:*** *39 children suffering from wheat harvest period (mid April - mid May) respiratory allergy along with randomly selected controls.* ***Methods:*** *Aerobiological surveys were done from March to June for identification of prevailing allergens for performing allergy tests. Patients were subjected to skin tests (ST), nasal provocation tests (NPT) and bronchial provocation tests (BPT) with extracts prepared from identified pollens and fungal spores. Specific IgE (SIgE) was assayed by ELISA and comparison between preseason, season and post-season values made.* ***Results:*** *81% patients had ST positive to antigen of wheat threshing dust (WTD), 30% to fungal antigens, 14% to wheat dust antigens and none to the wheat plant (WP) antigens. Nasal provocation test and bronchial provocation tests were also positive to WTD in 80% and 66% patients, respectively. WTD SIgE was demonstrated in 77% of ST positive patients.* ***Conclusions:*** *These in vivo and in vitro tests confirm wheat threshing dust as a major causative inhalant allergen for the April-May nasobronchial allergy; in addition, fungal allergens also play a role in l/3rd of these patients.*

Key words: *Wheat threshing dust allergen, Nasobronchial allergy.*

ASTHMA is by far the commonest of all chronic diseases of childhood. It is clearly a major problem leading to absenteeism from school, emergency room visits and hospitalizations. In children, nasobronchial allergy (NBA) is by and large extrinsic in nature (caused by allergens in patients' environment). The symptoms usually occur or exacerbate in a particular season except when the allergen exists year round, *e.g.,* house dust mite, dog and cat allergens, *etc.(I).* Seasonal allergy is common in children attending the Allergy Clinic of the Postgraduate Institute of Medical Education and Research, Chandigarh.

Seasonal symptoms were noted in many children in mid April-mid May. Kumar(2) had studied a small number of such patients who had related their symptoms to wheat threshing even though most of them were not involved in agriculture nor lived near the farms. They failed to react to skin tests (ST) done with the usual allergens present in environment during April-May with the exception of some fungal antigens but reacted to the wheat threshing dust (WTD) extract. It was postulated that some component(s) of WTD were responsible for this seasonal NBA. A systematic study was, therefore, undertaken to identify the

role of different wheat biosphere related agents in April-May NBA in children to identify the allergen(s) responsible for it.

Subjects and Methods

Thirty-nine patients in the age group of 6 to 17 years, who developed asthma and or rhinitis during wheat threshing period (mid April to mid May) for at least the past two consecutive seasons, formed the study group. An equal number of age, sex and rural/urban environment matched children who suffered from asthma and/or rhinitis in months other than April-May were taken as controls. A random selection was made. A detailed clinical proforma was filled on each child and consent was obtained.

For selection of specific allergens related to the patients environment, aerobiological surveys were done from beginning of March to end of June for three consecutive years in Chandigarh and surrounding villages considering month of March as preseason, April-May as season and June as the post-season period.

Roof top sampling was done at unobstructed sites of high buildings using open Petriplate exposure method(3). Petriplate with Rose Bengal Agar medium was used and simultaneous slide exposure was carried out as recommended by the National Survey Committee of the American Academy of Allergy(4).

For study of wheat biosphere, wheat plants (WP) were collected in green and hydrated state from different fields, homogenized and cultured for fungi which were stored at —20°C for preparation of extracts for allergy tests. Wheat threshing dust (WTD) was collected from different fields and cultured and fungi in 2% concentration in distilled water were stored as explained above. Indoor and outdoor surveys were done in wheat biosphere using Andersons Volumetric Sampler(5), open petriplate exposure and simultaneous slide exposure technique(4).

The houses of study group subjects as well as those of controls were studied. Subjects in both the groups were given individual sample collector(6) with a jelly smeared slide to be worn on their collars to catch the aerospora coming in their breathing zone throughout the period of their activity as well as at rest. Petriplates were also exposed in their bedrooms.

Allergen extracts were prepared from the WTD, WP and from the predominant fungi indentified from all the surveys. The allergens were prepared by the modified method of Agarwal *et al*.(7). The seasonal pollen extracts were obtained from Center for Biochemicals Technology, Delhi.

Intradermal skin tests (ST) were performed on volar aspect of the forearm using standard technique(8). Histamine and saline diluent were used as positive and negative controls, respectively.

Provocation tests (PT) were done in the asymptomatic period in patients making sure that they had not suffered from upper or lower respiratory infections in the preceding three weeks. Nasal provocation tests (NPT) and bronchial provocation tests (BPT) were done as described by Kumar(9) depending upon the target organ involved.

For specific IgE (SIgE) assay, 5 ml venous blood was collected in the months of January, May and August. Serum was stored at — 70" C in three aliquots. SIgE was assayed by ELISA technique described by Ali *et al*.(10). All tests were performed in duplicates. Comparisons between pre-season, season and post-season values were made. Ethical clearance was obtained from the Institute Ethical Committee and in accordance with that, informed consent was obtained from parents for performing the skin tests and provocation tests.

Analysis of variance test was applied for calculating the fungal recoveries in the three years under study. Analysis of variance was also used for pre-seasonal, seasonal, and post-seasonal SIgE comparison. Chi square test was used to see the significance of an allergen against the other allergens. Students Y test was used to compare results between two groups of subjects studied.

Results

Of the 39 children with respiratory allergy, 11 suffered from asthma, 13 from asthma and rhinitis and 15 from only allergic rhinitis. Twenty one children were from urban areas and eighteen were living in villages. Interestingly the appearance of symptoms and improvement were perceived by parents as a date related phenomenon. For example, in 1987 the wheat threshing was postponed because of unexpected rains in April. The wheat threshed was far less. Correspondingly, symptoms appeared late and lasted for shorter duration inspite of high fungal spore recoveries. In 1988, the symptoms appeared from mid April to mid May.

The predominant fungi identified from aerobiological surveys during different years are shown in *Table I.*

Type I or IgE mediated skin reaction to WTD extract was obtained in 32 (82%) children. Of the 7 children who did not react to WTD, 4 patients had positive ST to fungi. Overall 12 (30%) patients had ST positivity to fungi and 4 (14%) to wheat dust (WD). None responded to wheat plant and pollen extracts. In control subjects, WTD ST was positive in 3 (7%).

Of those with positive ST to WTD, 66.8% had BPT positive (a representative test is shown in *Fig. 1)* and 80% had NPT positive. BPT was positive in 36.3% and NPT was positive in 100% of patients who had positive ST to fungal extracts. Of the 4 children with ST positive for WD, none had positive NPT and only 1 had positive BPT.

Also, WTD SIgE was demonstrated in 77% of ST positive patients *(Fig 1).* A clear cut seasonal rise was noted over the preseason values. The difference of post-

TABLE I-*Fungi Recovered From Aerobiological Surveys*

Name of fungi	Air sampled
Aspergillus sp.	Wheat biosphere
Alternaria sp.	Wheat biosphere
Helminthosporium	'Wheat biosphere
Penicillium sp.	Indoor air
Aspergillus sp.	Indoor air
Rhizopus sp.	Indoor air
Alternaria sp.	Indoor air
Alternaria sp.	Breathing zone
Cladosporium sp.	Breathing zone
Alternaria sp.	Roof top air
Cladosporium sp.	Roof top air

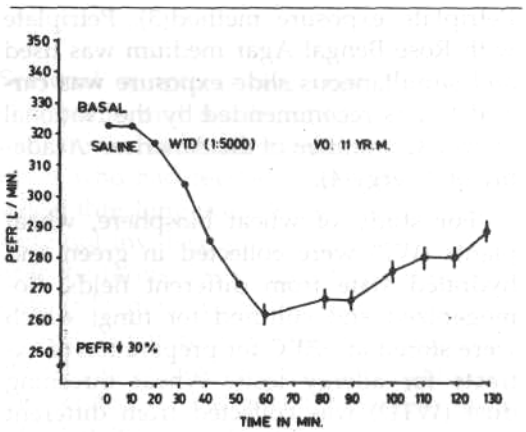

Fig. 1. A positive BPT obtained from an 11 year old male child showing a drop of more than 30% after inhalation of WTD extracts of 1:5000 (W/V).

seasonal and seasonal values was not significant *(Table II).* In controls, SIgE was not demonstrable. With regards to fungal antigens, the SIgE was assayed only against *Alternaria* sp. and *Aspergillus flavus* as these fungi contributed maximum to ST and provocation test positivity (23% and 18%, respectively).

Discussion

The results of this study assign a definite role to WTD as the major causative inhalant allergen for wheat harvest related NBA and it is the first report where IgE mediated WTD hypersensitivity (sensitization) has been confirmed based on *in vivo* and *in vitro* tests.

The overall ST positivity was 81% with WTD while SIgE was detected in *77%.* The possible explanation for higher percentage of ST positivity as compared to SIgE positivity could be due to the presence of irritant non allergenic substances in crude WTD extract(ll). Such non specific skin tests with crude grain dust extracts, Kapok cotton extracts, ragweed antigen and other extracts have been reported(12-14). The corroboration between ST and BPT positivity was not 100%.

TABLE II–*Wheat Threshing Dust Specific IgE (ELISA) Estimated Before, During and After the Season.*

Subjects	WTD Specific IgE		
	Preseason	*Season*	*Postseason*
Patients (mean ± SD)	0.34 ± 0.062	0.99 ± 0.132	0.61 ± 0.183
Control (mean ± SD)	–	0.03 ± 0.06	–

This could be due to sensitivity variation of the target organs. Presence of allergen specific IgE in blood may not imply the presence of same antibodies in adequate concentrations at mucosal surface. Had we proceeded with higher concentration (1: 50 W/V) of the allergen we may have obtained more positive BPT's as the concentration of allergen to elicit a positive skin test is much less than that needed to obtain a positive bronchial challenge(15, 16). The corroboration between ST, BPT, and NPT has been found to be variable amongst different antigens. Harris found a 78-90% correlation with dust, 64-90% with fungi and 60% with ragweed(17,18). Studies showing variable correlation between ST, PTs and SIgE are on record(19,20). The fact that WTD SIgE was demonstrable and ST and PT were positive, suggests that WTD indeed evoked the IgE mediated allergic response.

The ST positivity to fungi was found in l/3rd of children (13/39). In them (13 patients) the BPT and NPT to fungi was positive in 37% and 100%, respectively. The positive ST to fungal extracts have varied considerably in different studies ranging from 3-4% to about 80%(13,18). *Alternaria* and *Aspergillus* contributed to maximum ST, PT, SIgE positivity and were also recovered from wheat biosphere and breathing zone surveys in our study.

It is concluded that wheat threshing dust is a major causative inhalant allergen for the April-May NBA in this region; in addition, fungal allergens also play a role in 1/3 of these patients.

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