RESEARCH PAPER

Aeroallergen Sensitization in Childhood Asthmatics in Northern India

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Objective: To determine the prevalence of sensitization to common aeroallergens in asthmatic children and study the differences in characteristics of atopics and non atopics.

Design: Analysis of data from a prospective cohort study.

Setting: Pediatric Chest Clinic of tertiary care center in Northern India

Patients: Asthmatic children from 5-18 year of age.

Main outcome measures: Prevalence of sensitization to common aeroallergens.

Results: Skin prick testing (SPT) was performed on 180 children above 5 years of age, with a mean (SD) age of 111.4 (34.2) months. 100 children (55.6%) were sensitized to at least one aeroallergen, suggesting atopy; 68 (37.8%) were sensitized to more than one allergen. 36.7% children were sensitized to housefly antigen; 31.1% to rice grain dust, 18.3% to cockroach, and 7.8% to house dust mite antigens. Atopic children had significantly higher median FENO during follow up than non-atopic children (17.5 ppb *vs* 13 ppb, *P*=0.002). There was a positive correlation between age and the number of allergens that an individual was sensitized to (r= 0.21; *P*=0.0049).

Conclusions: More than half of asthmatic children in our cohort had sensitization to one or more aeroallergens suggesting atopy; sensitization was most commonly seen to housefly antigen and rice grain dust. Atopic children had significantly higher FENO measurements during follow up as compared to non-atopic children.

Key words: Aeroallergens, Atopy, Skin prick testing.

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Atopic (or allergic) asthma is a phenotype of asthma, which is characterized by allergic sensitization. Children with atopic asthma typically have onset early in life, have a positive family history of asthma or allergy, may have other coexistent allergic diseases (atopic dermatitis, allergic rhinitis), produce IgE specific to identifiable allergens, and have asthma exacerbation triggered by these allergens [1].

Aeroallergen sensitization is a risk factor in the development of childhood asthma, and most commonly implicated allergens are house dust mite (HDM), cockroach, and furred animals. Aeroallergen sensitization can be evaluated using either skin testing or measuring specific IgE to these aeroallergens. Skin prick testing (SPT) is an easy, cost-effective and convenient approach to identify sensitization to allergens. SPT detects the presence of allergen specific IgE bound to mast cells by eliciting mast cell degranulation to the specific allergen being tested [2].

Children with allergen sensitization are likely to have severe asthma, poorer lung function, and severe exacerbation due to these allergens [3,4]. The purpose of this study was to determine the prevalence of sensitization to common aeroallergens and study the differences in characteristics of atopics and non-atopics.

METHODS

This study was conducted at a tertiary care center in northern India. We are following a cohort of pediatric asthma patients (up to 18 yrs) from the Pediatric Chest Clinic since August 2009. Eligibility criteria were children with asthma who stayed in Delhi and nearby areas and willing to follow up 3 monthly regularly for at least 1 year. The diagnosis and treatment of asthma was based on the Global Initiative for Asthma (GINA) guidelines [5]. At enrolment, baseline data was collected, spirometry was performed [6], and FENO measurement was done [7]. Asthma was classified as per the NAEPP guidelines [8]. The patients were followed up every 3 months, symptom diary was maintained, and control was assessed as per GINA guidelines [5]. The cohort included 243 asthmatic children. As SPT needs cooperation of the child, we

performed SPT on children who were above five years of age. Of the cohort of 243 children, 180 children were 5 or more years of age and gave consent to SPT.

SPT was done using 12 aeroallergens. Saline was taken as negative control and histamine was used for positive control. Patients were not on antihistamines for at least 48 hours preceding the test. The twelve allergens tested were rice grain dust, wheat threshing dust, housefly, dog dander, female cockroach, house dust mite (*Dermatophagoides farinae*), *Curvularia lunata*, *Aspergillus tamari*, *Alternaria tenius*, *Prosopis juliflora*, *Cynodon dactylon*, and *Holoptelea integrifolia*. Allergens were obtained from All Cure Pharma Pvt Ltd, Bahadurgarh, Haryana. As it was not feasible for us to use a large panel, we used aero-allergens which we suspected to be of relevance considering our patient population (urban, rural, urban slum) and other previous studies from our country. Test was considered positive if wheal in any of the allergens was 3 mm or more than the negative control. Child was considered atopic if he demonstrated positive result to one or more allergen, and non-atopic if he had a negative SPT.

Study protocol was approved by Institutional Ethics committee of All India Institute of Medical Sciences.

Statistical analysis: Data were entered using Microsoft Access. Statistical analysis was performed using Stata 9.0 statistical software (Stata Corp., College Station, TX, USA). Differences between normally distributed variables were tested using unpaired Student's t-test whereas non-normally distributed variables were tested with two – sample Wilcoxon rank-sum (Mann-Whitney) test. Chi-square test was used for testing difference in proportions for categorical variables. *P* value of <0.05 was considered significant.

RESULTS

Skin prick testing was performed on 180 children above 5 years of age (75.6% males), with a mean age 9.2 ± 3.0 years. Baseline characteristics of the cohort are given in ***Table* I**. One hundred children (55.6%) were sensitized to at least one aeroallergen, suggesting atopy. Of the sensitized children, 49% had moderate persistent asthma, 38% had mild persistent asthma, 12% had severe persistent asthma, and 1% had intermittent asthma. The most common prevalence of aeroallergen sensitization was to housefly (36.7%) (**Table II**).

The characteristics of atopics and non-atopics are given in ***Table* III**. 155 children out of 180 were able to perform FENO appropriately as per guidelines [7]. There was a tendency towards lower median FENO at baseline in the non-atopic group (*P*=0.052). During follow up, median FENO was significantly lower in the non atopic group (*P*=0.002); however, this difference was not observed during acute exacerbation. There was a positive correlation between age and the number of allergens that an individual was sensitized to (r= 0.21; *P*=0.0049) (***Fig.* 1**).

DISCUSSION

In our study, 55.6% children of our cohort were sensitized to one or more aeroallergens. Atopy and allergen exposure are known to exacerbate asthma and atopy is a risk factor for relapse of asthma after remission [9]. Prevalence of atopy (defined as at least one positive SPT) in childhood asthmatics varies from 45 to 79% and percentage of asthma cases attributable to atopy in population based studies varies from 25 to 63% [10].

We used a panel of 12 aeroallergens for skin testing. Sensitization to housefly and rice grain dust was the commonest. There are few studies which have evaluated insect sensitization in asthma patients [11-13]. Rice grain dust is also an uncommon cause of respiratory allergy [14]. Although these are infrequent cause of allergic sensitization, it won't be prudent to dismiss them as just irritation than sensitization.

The sensitization pattern observed in our study is different from other studies from the same geographical region. An earlier study from North India which assessed

TABLE I BASELINE CHARACTERISTICS

Characteristics	*n (%)*
Baseline asthma severity	
Intermittent asthma, *n* (%)	4 (2.2)
Mild persistent asthma, *n* (%)	61 (33.9)
Moderate persistent asthma, *n* (%)	96 (53.3)
Severe persistent asthma, *n* (%)	19 (10.6)
Age of onset of symptoms (*n*=177)	35.0 (35.7)
Exposure to tobacco smoke at home, (%)	70 (39.6)
Atopy (skin prick testing, *n*=180)	
Negative, *n* (%)	80 (44.4)
Positive to at least one allergen, *n* (%)	100 (55.6)
Positive to more than one allergen, *n* (%)	68 (37.8)
Residence	
Rural, *n* (%)	43 (24.0)
Urban, *n* (%)	130 (72.6)
Urban slum, *n* (%)	6 (3.4)
Family history of asthma, *n* (%)	85 (47.8)
Family history of any allergic disease	112 (62.9)

TABLE II AEROALLERGENS CAUSING SENSITIZATION

Aeroallergen	*n (%)*	*Age, mean (SD)*
Housefly	66 (36.7)	124 (34.3)
Rice grain dust	56 (31.1)	115.6 (35.2)
Female cockroach	33 (18.3)	116.5 (35.4)
Curvularia lunata	27 (15.0)	113.7 (30.0)
Aspergillus tamari	26 (14.4)	125.1 (30.1)
Alternaria tenius	18 (10.0)	119 (32.2)
Dog dander	14 (7.8)	119.3 (34.2)
House dust mite	14 (7.8)	123.9 (37.5)
Cynodon dactylon	12 (6.7)	127.6 (38.0)
Holoptelea integrifolia	12 (6.7)	124.3 (31.6)
Wheat threshing dust	12 (6.7)	120.3 (35.4)
Prosopis juliflora	3 (1.7)	131.3 (45.2)

480 asthmatics/allergics found *Prosopis juliflora* among pollen and *Alternaria alternata* as important sensitizers with 34.7% and 17.7% skin positivity, respectively [15]. Another study from Southern India in patients with nasobronchial allergy showed high prevalence of mite allergy (73.7%) and pollen allergy (75.8%) [16]. The reason for this difference from same geographical area is probably because of seasonal and annual fluctuations in allergens. Heterogeneity in allergen extract composition can possibly lead to the different pattern of sensitization observed in our study [17]. The variable composition and content of allergenic extract of different manufacturers may affect the allergenicity of the extract [18].

Sensitization to house dust mite has been incriminated in the development of asthma, and has been observed in over 50% children and adolescents [19].

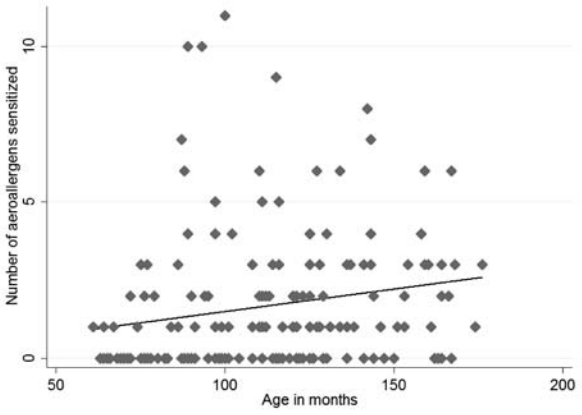

FIG. 1 *Correlation between age and the number of aeroallergen sensitized.*

Sensitization to allergens (mite, dog or cat) in the first 3 years of life is associated with loss of lung function at school age [20]. A whole population birth cohort study identified house dust mite as the most common allergen [21]. Dust mite allergy has also been associated with increased asthma morbidity and severity [22]. Sensitization to house dust mite was observed in 7.8% patients in our cohort. The low incidence of sensitization to house dust mite in our study is surprising given the high prevalence of sensitization in studies from the developed countries. There is limited data on house dust mite allergy in asthmatic children in India. A study from Mysore in children and adults with allergic rhinitis and/or asthma found dust mite allergy in 65-70% [23].

Our study showed a positive correlation between age and the number of allergens that an individual was sensitized to. In a recent birth cohort study, it was seen that allergen sensitization continued to increase over childhood and adolescence, and presence of sensitization at 4 years predicted later sensitization to additional allergens [24].

Evidence on the relation between asthma severity and sensitization or atopy is conflicting [25-28]. In our study, asthma severity was not different between atopics and non-atopics. This can be explained by the fact that acute exacerbation and loss of asthma control can be caused by a number of factors other than allergen exposure.

Non-atopic asthmatics were younger than atopic asthmatics, and had a trend towards younger age of onset of asthma. Similar findings have been reported from a hospital based study from Spain (29). Other characteristics like ICS use, acute exacerbation episodes per child per year, and PFT measurements were similar in the two groups.

Allergen sensitization has been known to cause increased FENO, not only in children but also adults [30-32]. FENO has been found to be elevated not only in asthmatics but also other atopic conditions like allergic rhinitis and atopic eczema. Atopic individuals have allergic inflammation and the allergological markers (BAL eosinophils, blood eosinophils, eosinophils in bronchial biopsies) are known to correlate with FENO. The exact reason for high FENO in eosinophilic inflammation is not clear, however there could be a possible role of NOS 2 upregulation. Similarly, in our study, FENO was significantly higher in atopic children as compared to non atopic children at follow up (P=0.002). However, there was no difference in FENO at baseline and during acute exacerbation in the two groups. The reason why the difference at baseline was not observed may be because of the fact that at enrolment the patients were

TABLE III COMPARISON BETWEEN ATOPIC AND NON-ATOPIC ASTHMATICS

	Atopic (n=100)	*Non-atopic (n=80)*	*P value*
Mean age, years (SD)	9.8 (2.9)	8.3 (2.9)	0.0009
Males, n (%)	80 (80)	56 (70)	0.121
Mean weight, kg (SD)	29.1 (10.1)	22.4 (8.5)	<0.0001
Mean height, cm (SD)	132.0 (19.6)	122.1 (29.1)	0.007
Residence			
Urban, *n* (%)	25 (22.8)	18 (25.0)	0.796
Urban slum, *n* (%)	71 (74.7)	59 (71.0)	
Rural, *n* (%)	4 (2.5)	2 (4.0)	
Baseline asthma severity			
Intermittent, *n* (%)	1 (1)	3 (3.8)	0.276
Mild persistent, *n* (%)	38 (38)	23 (28.8)	
Moderate persistent, *n* (%)	49 (49)	47 (58.8)	
Severe persistent, *n* (%)	12 (12)	7 (8.8)	
Family history of asthma, *n* (%)	42 (42.4)	43 (54.4)	0.111
Family history of asthma or allergy, *n* (%)	58 (58.6)	54 (68.4)	0.186
Baseline FEV1, % predicted, mean (SD)	85.2 (19.8) *n*=92	85.8 (17.8) *n*=65	0.826
Baseline PEF, % predicted, mean (SD)	74.5 (22.2) *n*=92	68.2 (22.2) *n*=69	0.076
Baseline FENO, median (IQR), ppb	18 (10-30) *n*=91	14 (10-21.5) *n*=64	0.052
Mean FENO during follow up, median (IQR), ppb	17.5 (11.7-27.5) *n*=93	13 (9.7-17.5) *n*=77	0.002
Mean FENO during exacerbation, median (IQR), ppb	18 (13-25.5) *n*=42	14 (10-22) *n*=27	0.202
Number of acute exacerbations per child per year, mean (SD)	0.86 (1.63) *n*=95	0.63 (1.26) *n*=77	0.319
Age of onset of symptoms (mo), mean (SD)	39.2 (40.0)	29.3 (28.7)	0.059
Daily dose of inhaled corticosteroids (μg), mean (SD)	580.0 (226.2)	573.0 (224.7)	0.841

heterogeneous in terms of asthma control and steroid use. This study corroborates the fact that atopic asthmatics have higher FENO, but whether this helps to identify a clinically relevant phenotype of asthma, has to be studied [33].

Few studies are available from the Indian subcontinent which have evaluated prevalence of allergic sensitization in childhood asthma and also FENO in relation to allergic sensitization. The cohort is being followed up to evaluate the natural history of atopic asthma and allergic sensitization. One limitation of our study is that the panel of aero-allergens used may have been inadequate and a larger panel should be used to thoroughly evaluate the aero-allergens in our geographic region. The study was conducted in a hospital and not in the general population, so inherent sampling bias cannot be ruled out and therefore, our results may not be generalizable. In subjects with a negative SPT, further additional panels of allergens were not tested and therefore, true non-atopy could not be confirmed.

The pattern of alloallergen sensitization in this study is not similar to earlier studies from this or other geographical regions. Atopic children had significantly higher FENO measurements as compared to non-atopic children. There is a need of know about an optimal panel of aeroallergens which can be only done by studies using a larger panel of aeroallergens.

Contributors: AA, RL and SKK conceived and designed the study, analyzed the data and were directly involved in paper writing; RL will act as guarantor; DR, AP, were responsible for data collection; DR and AM contributed to analysis and drafting of the paper; New Delhi childhood asthma study group participated in development of study.
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WHAT IS ALREADY KNOWN

- Aeroallergen sensitization is a risk factor in the development of childhood asthma.

WHAT THIS STUDY ADDS

- Close to half of asthmatic children have sensitization to one or more aeroallergens suggesting atopy.
- Sensitization is most commonly seen to housefly antigen and rice grain dust.

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