

Exploring the effect of presence and type of allergen sensitization on fractional exhaled nitric oxide, immunoglobulin E, and interleukins 4, 5, and 13 among asthmatics

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ABSTRACT

Objective: Asthma is associated with airway inflammation. Allergen sensitization (atopy) is common in asthma. This study explored the effect of food and/or aeroallergen sensitization; on airway and systemic inflammation using fractional exhaled nitric oxide (FE_{NO}) and interleukins (ILs) 4, 5, and 13. **Methods:** The study enrolled asthmatics (diagnosed using Global Initiative for Asthma guidelines). Atopy was diagnosed using skin-prick testing (SPT). All subjects underwent testing for FE_{NO}, blood absolute eosinophil count (AEC), and serum levels of immunoglobulin E (IgE), and ILs 4, 5, and 13. Asthmatics (BA) were classified as atopic (BA-A) and nonatopic (BA-N). Atopic-asthmatics were subclassified as exclusively food allergen (AtAA) or aeroallergen (AtAA); or dually (AtFAA) sensitized. **Results:** The study enrolled 203 asthmatics (BA) and 50 controls. Among BA, 169 were BA-A and 34 were BA-N. Mean values of AEC, serum IgE and FE_{NO}, and ILs 4 and 13 were significantly higher in BA-A than BA-N (659 vs. 218/mm³, 638 vs. 217 IU/ml, 39.2 vs. 20.0 ppb, 14.96 vs. 8.04 pg/ml, and 22.12 vs. 11.64 pg/ml, respectively). Meanwhile, mean IL-5 was higher in BA-A (11.01 vs. 8.76 pg/ml; *P* = 0.22), but not statistically significant. Subgroup analysis of atopic asthmatics (i.e., AtFA, AtAA, and AtFAA), revealed similar mean values for FE_{NO} (31.99 vs. 40.16 vs. 39.46 ppb), AEC (691.00 vs. 653.07 vs. 659.88/mm³), IgE (635.60 vs. 630.32 vs. 646.39 IU/ml), IL-4 (12.63 vs. 14.74 vs. 15.44 pg/ml), and IL-13 (18.38 vs. 19.87 vs. 24.57 pg/ml). No difference was observed among subgroups of atopic-asthmatics. **Conclusion:** Subgroups of atopic-asthmatics did not show any consistent difference across all the studied parameters.

KEY WORDS: Aero allergen, atopic, biomarkers, food allergen, nonatopic

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INTRODUCTION

Asthma is a global health problem with prevalence rates reported between 1% and 18% in different countries.^[1] In India, the prevalence of asthma is estimated to be 2.05% as per the Indian

Study on Epidemiology of Asthma, Respiratory symptoms, and Chronic bronchitis (INSEARCH).^[2] Meanwhile, the prevalence in Indian adolescents is estimated at 2.9% (GINA).^[1]

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Asthma is often clustered on the presence or absence of atopy.^[3,4] Atopy is defined as the genetic predisposition of an individual to produce high quantities of immunoglobulin E (IgE) in response to an allergen.^[3,4] Skin prick tests (SPTs) using allergen extracts can identify an individual's sensitization to an allergen,^[1,3] similar to quantitative serum-specific IgE analysis.^[1,5,6] Exposure to allergens can occur in several ways, of which the inhaled route (aero allergens [AAs]) and the ingested route (food allergens [FAs]) are most common.^[7-10]

Airway inflammation is a key pathological feature in asthma.^[1] The inflamed airways in asthma have been found to show increased levels of nitric oxide (NO) secondary to inducible NO synthase.^[1,11,12] The measurement of this fractional exhaled NO (FE_{NO}) in asthmatic individuals is used as a noninvasive marker of eosinophilic airway inflammation.^[11-13] Interleukins (ILs) are signaling molecules secreted by a variety of body cells. ILs 4, 5, and 13 are secreted from key cells of allergic inflammation; like T cells and mast cells, and in the case of IL-4, additionally basophils and eosinophils.^[14,15] These ILs play a central role in immune-regulation of the Th2-mediated response seen with allergen exposure and have been found in increased levels in bronchoalveolar lavage (BAL), sputum and peripheral blood^[15,16] in atopic asthma.

FA sensitization is associated with increase in asthma symptoms^[9] and medication use.^[10] Recently, it has been reported, that sensitization against FAs is independently associated with increased airway hyper responsiveness in patients suffering from asthma.^[17,18] A study comprising 411 asthmatic patients, found a positive correlation between FE_{NO} and FA specific serum IgE levels.^[17]

The nature of exposure for “ingested” allergen is different from an “inhaled” one. The objective of this study is to look for a possible difference in inflammatory markers (i.e., FE_{NO}, serum IgE and Th2 ILs: 4, 5, and 13) in FA and AA sensitized asthmatics.

The influence of food sensitization on inflammatory markers in asthma has not been well established in Indian patients previously. This study attempts to establish a relationship, if any, between sensitization to food and aeroallergen and airway and systemic inflammation in asthma in India.

MATERIALS AND METHODS

Study design

The present study was a cross-sectional analysis conducted over a period of 2 years at the Department of Pulmonary Medicine at Vallabhbhai Patel Chest Institute, New Delhi (India), after an approval by the Institutional Ethical Committee. Individuals aged 18–40 years were enrolled after written informed consent. The diagnosis of asthma was made in accordance with GINA guidelines,

2012.^[11] The exclusion criteria were current or former smoker, respiratory tract (upper or lower) infection in previous 1 month, oral steroids in preceding 1 month, pregnant or lactating patients, inability to perform FE_{NO} measurement or spirometry. In addition, for the purpose of enrolling controls, healthy individuals from the general population were approached. Those who volunteered were enquired for personal and family history of asthma, atopy, or any chronic respiratory illness. Only those individuals with negative answers to all of the aforementioned were enrolled as controls in the study.

Spirometry

Using a dry, rolling-seal spirometer of the Benchmark model lung function machine (P.K. Morgan, Kent, UK.), flow volume and volume time curves were obtained as per the ATS recommendations.^[19] The test was repeated 15 min after inhalation of 200 µg salbutamol for reversibility testing.

Measurement of fractional exhaled nitric oxide

All subjects underwent measurements of exhaled NO using NIOX chemiluminescence analyzer (Aerocrine AB, Sweden) in accordance with ATS/ERS recommendations.^[12] A mouthpiece was inserted in the mouth, and the subject was asked to inhale maximally to total lung capacity, followed immediately by exhalation at a constant rate of 50 ml/s, being monitored on screen, without breath holding or jerky respiration. Repeat testing was done to obtain two plateau values with a variation of 10% or less. The mean value was taken as the measurement.

Serum total immunoglobulin E, interleukins, and blood eosinophil levels

Serum total IgE was estimated in all the subjects using sandwich enzyme immunoassay (MINILYSER-TECAN, Calbiotech) kit as per the manufacturer's instructions. ILs 4, 5 and 13 were measured using commercially available, standardized quantitative ELISA kits from Diaclone. Eosinophil counts were done on venous blood by automated analyzer using EDTA anticoagulant.

Skin prick test

SPT was performed as per the Indian guidelines,^[20] against 67 FAs and 67 common AAs in all list of allergens used in SPT is given in Supplementary file 1. For the purpose of SPT, 67 preselected food items to which we have observed positive reactions in daily practice, were used.^[20] A drop of allergen (All Cure Pharma Pvt., Ltd., Delhi, India) was put on volar aspect of forearm, and then site was skin pricked with a 26 G hypodermic needle at 45° angle. A positive result to an allergen was indicated by mean diameter measuring 3 mm or greater than the negative control of buffer saline. In this study, atopy was defined as a positive SPT result to at least one allergen.

Individuals testing positive for at least one allergen during SPT were taken as atopic. Asthmatic individuals (BA) were classified into atopic (BA-A) and nonatopic (BA-N).

Atopic asthmatics (BA-A) were further subgrouped on the basis of their sensitization pattern as exclusively FA atopic (AtFA), exclusively AA atopic (AtAA), or both FA and AA atopic (AtFAA).

Statistics

The data analysis was performed using SPSS (Chicago, IL, USA). Descriptive statistics in the form of mean and standard deviations or proportions were used to characterize the study sample. For quantitative data, the difference between the means of the two groups was compared by *t*-test (for normal distribution) or Mann-Whitney test (for nonnormal distribution). For qualitative data, Chi-square or Fischer's exact test was used to observe the difference between proportions for independent groups. *P* < 0.05 was considered statistically significant.

RESULTS

Patient flow for the study is depicted in Figure 1. A total of 253 individuals were fully evaluated, including 50 healthy

Table 1: Population characteristics and study results for asthmatic individuals and healthy controls

	Asthmatics (BA)	Controls (C)	<i>P</i>
<i>n</i>	203	50	
Age (years)	26.5±6.38	29.1±6.22	NS
Male/female (<i>n</i>)	97/106	28/22	NS
BMI (kg/m ²)	23.6±4.2	24.5±3.9	NS
FEV ₁ /FVC %	76.48±12.07	88.74±8.36	<0.001
FEV ₁ % pred	82.67±17.76	95.18±8.84	<0.001
FVC % pred	92.96±14.47	100.98±8.75	<0.001
AEC (/mm ³)	584±272	119±91	<0.001
serum total IgE (IU/ml)	568±256	125±69	<0.001
FE _{NO} (ppb)	36.1±26.5	12.9±11.1	<0.001
IL 4 (pg/ml)	13.8±9.7	8.34±7.24	<0.001
IL 5 (pg/ml)	10.6±6.74	7.34±3.2	<0.001
IL 13 (pg/ml)	20.3±18.08	7.61±5.35	<0.001

BMI: Body mass index, FEV₁% pred: Forced expiratory volume in first second (percentage predicted), FVC % pred: Forced vital capacity (percentage predicted), AEC: Blood absolute eosinophil count, FE_{NO}: Fractional exhaled nitric oxide, IL: Interleukin, NS: Not significant

controls (C). Of the 203 asthmatics (BA) enrolled for the study, 169 (83.25% asthmatics) were atopic (vis-à-vis 44% of controls). The demographic details and results from the test for clinical significance (one-way ANOVA) from each group and subgroup are given in Tables 1-3.

Allergen sensitization (atopy)

Among atopic asthmatics (BA-A), the prevalence of exclusive FA sensitization was 5.92% (AtFA, *n* = 10), exclusive AA sensitization was 44.38% (AtAA, *n* = 75) and 49.70% were sensitized to both FA and AAs (AtFAA, *n* = 84). Taking all asthmatics (BA) enrolled in the study into account, these prevalence rates are 4.93%, 36.94%, and 41.38% for AtFA, AtAA, and AtFAA, respectively; while the remaining 16.75% are nonatopic asthmatics (BA-N)

The asthmatics were found to be sensitized to 7.35 allergens on an average (range from 1 to 57 antigens). Furthermore, they were found to be sensitized to a mean of 1.87 FAs (range from 1 to 35) and 5.46 AAs (range from 1 to 42). In comparison, controls were sensitized to 0.30 FAs and 1.18 AAs. This distribution was found to be statistically significant with *P* < 0.05 (unpaired *t*-test).

Interleukins 4, 5 and 13

Patients with asthma had higher values of IL 4, 5, and 13 compared to healthy controls. Further, the presence of atopy in asthma was found to be associated with significantly higher levels of ILs 4 than an absence of atopy (14.96 ± 10.17 vs. 8.04 ± 3.78 pg/ml, *P* < 0.001). A similar trend was also observed for IL 13 (22.12 ± 18.98 vs. 11.64 ± 8.52 pg/ml, *P* < 0.001). IL 5 levels were found to be higher in atopic than nonatopic asthma (11.01 ± 6.62 vs. 8.67 ± 7.08 pg/ml, *P* = 0.224), though the difference was found to be not significant.

Comparison of AtFA, AtAA, and AtFAA

The characteristics of the study population and their clinical parameters are described in Table 3. No statistically significant difference was found in mean FE_{NO} values

Table 2: Population characteristics and study results in atopic asthmatics, nonatopic asthmatics, and healthy controls.

	Atopic (BA-A)	Nonatopic (BA-N)	Controls (C)	<i>P</i> [#] (OWA)	<i>P</i> [*] (BA-A vs. BA-N)	<i>P</i> [*] (BA-A vs. C)	<i>P</i> [*] (BA-N vs. C)
<i>n</i>	169	34	50				
Age (years)	26.2±6.2	27.88±7.01	29.1±6.22				
Male/female (<i>n</i>)	87/82	10/24	28/22				
BMI (kg/m ²)	23.6±4.2	23.7±3.9	24.5±3.9				
FEV ₁ /FVC %	76.45±11.92	76.64±12.99	88.74±8.36	0.001	1.000	<0.001	<0.001
FEV ₁ % pred	82.57±16.97	83.21±21.52	95.18±8.84	0.001	0.998	<0.001	0.011
FVC % pred	93.39±12.15	90.83±22.92	100.98±8.75	0.001	0.893	<0.001	0.053
AEC (/mm ³)	659±230	218±137	119±91	0.001	<0.001	<0.001	0.002
Serum total IgE (IU/ml)	638±214	217±127	125±69	0.001	<0.001	<0.001	0.001
FE _{NO} (ppb)	39.2±27.1	20±16.1	12.9±11.1	0.001	<0.001	<0.001	0.073
IL 4 (pg/ml)	14.96±10.17	8.04±3.78	8.34±7.24	0.001	<0.001	<0.001	0.993
IL 5 (pg/ml)	11.01±6.62	8.67±7.08	7.34±3.2	0.001	0.224	<0.001	0.667
IL 13 (pg/ml)	22.12±18.98	11.64±8.52	7.61±5.35	0.001	<0.001	<0.001	0.052

[#]*P* (OWA): *P* values as established by one-way ANOVA analysis, ^{*}*P* values using *post hoc* analysis by Dunnett T3 test are given in rightmost three columns marked *P* (BA-A vs. BA-N), *P* (BA-A vs. C) and *P* (BA-N vs. C). BMI: Body mass index, FEV₁% pred: Forced expiratory volume in first second (percentage predicted), FVC % pred: Forced vital capacity (percentage predicted), AEC: Blood absolute eosinophil count, FE_{NO}: Fractional exhaled nitric oxide, IL: Interleukin, OWA: One Way ANOVA analysis

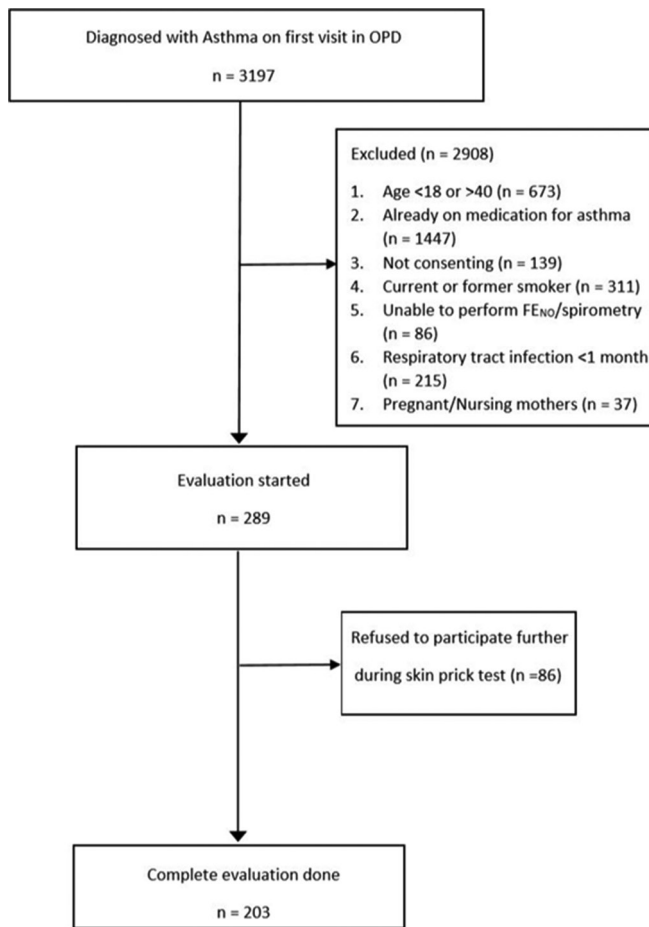


Figure 1: Patient flow diagram

Table 3: Population characteristics and study results in subgroups of atopic asthmatics based on sensitization to food allergens, aero allergens, and both food and aero allergens

	AtFA	AtAA	AtFAA	P
n	10	75	84	
Age (years)	28.8±5.45	25.8±6.52	26.31±6.04	
Male/female (n)	1/9	35/40	51/33	
BMI (kg/m ²)	26.3±7.31	22.6±4.05	24.1±3.90	
FEV ₁ /FVC %	71.24±13.82	78.26±10.59	75.46±12.64	0.120
FEV ₁ % pred	77.30±22.83	85.25±16.62	80.80±16.35	0.153
FVC % pred	92.10±14.55	94.08±11.78	92.93±12.92	0.790
AEC (/mm ³)	691±142	653±282	660±185	0.886
Serum total IgE (IU/ml)	635.60±204.51	630.32±257.98	646.39±168.99	0.894
FE _{NO} (ppb)	31.99±23.26	40.16±31.46	39.46±23.36	0.672
IL 4 (pg/ml)	12.63±7.18	14.74±10.01	15.44±10.65	0.690
IL 5 (pg/ml)	9.58±5.22	9.41±2.02	12.61±8.78	0.007
IL 13 (pg/ml)	18.38±6	19.87±15.73	24.57±22.18	0.242

P values as established by one-way ANOVA analysis. AtFA: asthmatics with exclusive food allergen sensitization, AtAA: asthmatics with exclusive aeroallergen sensitization, AtFAA: asthmatics with both food and aero allergen sensitization, BMI: Body mass index, FEV₁% pred: Forced expiratory volume in first second (percentage predicted), FVC % pred: Forced vital capacity (percentage predicted), AEC: Blood absolute eosinophil count, FE_{NO}: Fractional exhaled nitric oxide, IL: Interleukin

among AtFA, AtAA, and AtFAA groups ($P = 0.672$ on one-way ANOVA test). Serum levels of IgE, IL 4, and IL 13

also did not show statistically significant variations across the three subgroups, with P values of 0.894, 0.690, and 0.242, respectively. Serum IL 5 was higher (12.61 ± 8.78 pg/ml) in AtFAA compared to AtFA 9.58 ± 5.22 pg/ml and AtAA 9.41 ± 2.02 pg/ml sensitized individuals. P values on post hoc analysis were 0.005 for AtFAA versus AtAA, 0.999 for AtFAA versus AtFA and 0.333 for AtFA versus AtAA.

DISCUSSION

AAs have long been held responsible for eliciting airway hyper responsiveness in sensitized asthmatics. Recently, even FAs^[17,18] have been shown to produce a similar response. Identification of the presence or absence of allergen sensitization (or atopy) plays an important role in the management of asthma. In this study, we looked for a possible correlation between FA and/or AA sensitization with atopic airway and blood inflammatory markers. Further, we sought to look for possible variations between food and aeroallergen sensitized asthma in terms of markers of atopy and atopic inflammation.

The phenomenon of atopy, by SPT, was found in 83.25% asthmatics. Atopic asthmatics were sensitized to an average $8.83 (\pm 0.64)$ allergens. In a study on 751 individuals, Nieves *et al.*^[21] found atopy in 73.37% asthmatics. In the same study, lung function assessment by spirometry revealed significantly lower FEV₁/FVC and FEV₁ in the nonatopic group compared to the atopic group. In contrast, the present study found similar lung function values in the two groups. The slightly higher prevalence of atopy and a similar lung function between atopics and nonatopics is likely attributable to the exclusion of ever-smokers from the present study. Indeed, Nieves *et al.*^[21] reported significantly higher incidence (38.5%) of ever smoking in nonatopic asthmatics, and attributed this to the finding of worse lung function in this group. The effect of worse air pollution and higher suspended particulate matter,^[22,23] cannot be ruled out in the current study.

The prevalence of FA and AA sensitization in the present study was 46.30% and 78.3%, respectively. This is in agreement with a previous study by Kumar *et al.*,^[24] where AA sensitization was present in 449 of 613 (or 73.3% of) asthmatics.

Atopy was found to have a slight but significant male predilection in the current study. FA sensitization and AA sensitization individually were also significantly more prevalent in males. This is in line with findings from a previous study by Arbes *et al.*,^[25] which also showed atopy to be more common in males.

The immunological response after exposure to an immunogen (allergen or otherwise) can be broadly classified as Th1 predominant or Th2 predominant.^[26] The Th2 pathway, consisting of cells such as eosinophils, mast cells, and basophils, is the dominant response in allergen

sensitization.^[26] IgE is the Immunoglobulin central to the development of atopy.^[26] High levels of blood eosinophils and serum IgE were found in atopic asthmatics compared to both healthy controls and asthmatics without atopy. This is consistent with previous studies by Burrows *et al.*^[27] and Bousquet *et al.*^[28]

ILs are mediators of inflammation and play a central role in the pathogenesis of asthma. The chief inflammatory cytokines in Th2 response are ILs 4, 5, and 13. Recently, it was found that blood and BAL levels of ILs 4, 5, and 13 were raised in atopic asthmatics.^[16,29,30] Likewise, in the present study, these ILs were found in significantly higher quantities in the blood of individuals with atopic asthma.

FE_{NO} is a noninvasive, rapid, simple, and relatively inexpensive marker for airway inflammation and hyper responsiveness. Studies have shown a strong correlation of levels of FE_{NO} with eosinophilic inflammation and have proven its utility as a marker for sputum eosinophilia. Previously, Kumar and Gupta.^[31,32] had found a positive correlation of FE_{NO} with the presence of atopy in the Indian population. The current study is consistent with literature; FE_{NO} was significantly higher in atopic asthmatics than both nonatopic asthmatics and controls.

Sensitized individuals get exposed to their sensitizing allergens by a number of routes; inhalation, ingestion, skin contact, etc.^[7,8] The possibility of differences in clinical characteristics in asthmatic individuals with different sensitization patterns, i.e., food (ingested) and aero (inhaled) allergens is an intriguing facet that has not received much attention in the past.

In the present study, we assessed airflow limitation, blood eosinophil count, FE_{NO}, serum levels of IgE, and serum ILs 4, 5, and 13 to explore this facet among atopic asthmatics with sensitization exclusively to FAs (i.e. AtFA), exclusively to AAs (i.e. AtAA), and to both FA and AA (i.e. FAA).

The degree of airflow limitation (as assessed by spirometry), blood eosinophilia, and levels of serum IgE was similar in all three groups. Likewise, FE_{NO} levels were also raised irrespective of the type of allergen sensitization (ingested or inhaled). This is concurrent with a study done by Patellis *et al.*,^[17] who found a high levels of FE_{NO} in asthmatics sensitized to FAs independently from AAs.

Serum levels of ILs 4, 5, and 13 were also similarly high in across all three groups. The only outlier comparison was IL 5 being significantly higher in AtFAA compared to AtAA individuals. Here too, there was no significant difference in AtFA compared to either AtAA or AtFAA. Importantly, none of the three groups showed a consistent trend of higher values across all parameters.

The concept of FAs behaving like AAs is not entirely new. Roberts *et al.*^[33] identified 12 children with known

food allergy and asthma. They found that aerosolized FAs triggered asthma symptoms in these individuals. Unlike deglutition, chewing is not associated with cessation of respiration and/or closure of supra glottis. Thus, tiny particles of food may get inhaled into the airways. Another possible, though not mutually exclusive, mechanism may be the occurrence of an immune response in the digestive tract (upper and/or lower). The already primed airways in asthmatics may then show an exaggerated response or even by-stander effect.

The findings of our study are uniquely centered on atopy, i.e., allergen sensitization and not established allergy to FAs. We believe the present study is the first study done in India, involving multiple investigation based and objective parameters for comparison between FAs and AAs in atopic asthma. AA sensitization in asthmatics was not found to be associated with significantly different levels of ILs 4, 5, and 13, compared to food sensitization. As a corollary, we suggest that the final inflammatory response, located in the airway in case of asthma, in food sensitization is similar to aeroallergen sensitization. The effect in food sensitization on ILs, independent from aeroallergen sensitization, is a novel finding in this study. Interestingly, IL 5 was higher in asthmatics sensitized to both FA and AA simultaneously as compared to either FA or AA exclusively. Whether this finding carries an impact value is yet to be established.

CONCLUSION

The present study evaluated eosinophil counts, serum total IgE, and levels of local (FE_{NO}) and systemic inflammatory markers of TH2 response (IL 4, 5, and 13) in patients of asthma in the Indian context. These clinical parameters were found to be raised in asthma with atopy compared to asthma without atopy and healthy controls. Furthermore, among atopic asthmatics, these parameters were affected to a similar extent irrespective of the type of sensitization, i.e., FA, AA, and both.

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Conflicts of interest

There are no conflicts of interest.

REFERENCES

1. Global Initiative for Asthma (GINA). Global Strategy for Asthma Management and Prevention. Available from: <http://www.ginasthma.org>. [Last accessed on 2019 Dec 25].
2. Agarwal R, Denning DW, Chakrabarti A. Estimation of the burden of chronic and allergic pulmonary aspergillosis in India. *PLoS One* 2014;9:e114745.
3. Pearce N, Pekkanen J, Beasley R. How much asthma is really attributable to atopy? *Thorax* 1999;54:268-72.
4. Reed CE. The natural history of asthma. *J Allergy Clin Immunol* 2006;118:543-8.
5. Jarvis D, Burney P. ABC of allergies. The epidemiology of allergic disease. *BMJ* 1998;316:607-10.
6. Zimmerman B, Feanny S, Reisman J, Hak H, Rashed N, McLaughlin FJ,

- et al.* Allergy in asthma. I. The dose relationship of allergy to severity of childhood asthma. *J Allergy Clin Immunol* 1988;81:63-70.
7. Sporik R, Platts-Mills TA. Allergen exposure and the development of asthma. *Thorax* 2001;56 Suppl 2:ii58-63.
8. Kulig M, Bergmann R, Klettke U, Wahn V, Tacke U, Wahn U. Natural course of sensitization to food and inhalant allergens during the first 6 years of life. *J Allergy Clin Immunol* 1999;103:1173-9.
9. Zicari AM, Indinnimeo L, De Castro G, Zappalà D, Tancredi G, Bonci E, *et al.* Food allergy and the development of asthma symptoms. *Int J Immunopathol Pharmacol* 2012;25:731-40.
10. Björnsson E, Janson C, Plaschke P, Norrman E, Sjöberg O. Prevalence of sensitization to food allergens in adult Swedes. *Ann Allergy Asthma Immunol* 1996;77:327-32.
11. Barnes PJ, Dweik RA, Gelb AF, Gibson PG, George SC, Grasemann H, *et al.* Exhaled nitric oxide in pulmonary diseases: A comprehensive review. *Chest* 2010;138:682-92.
12. Dweik RA, Boggs PB, Erzurum SC, Irvin CG, Leigh MW, Lundberg JO, *et al.* An official ATS clinical practice guideline: Interpretation of exhaled nitric oxide levels (FENO) for clinical applications. *Am J Respir Crit Care Med* 2011;184:602-15.
13. Jatakanon A, Lim S, Kharitonov SA, Chung KF, Barnes PJ. Correlation between exhaled nitric oxide, sputum eosinophils, and methacholine responsiveness in patients with mild asthma. *Thorax* 1998;53:91-5.
14. O'Shea JJ. Jaks, STATs, cytokine signal transduction, and immunoregulation: Are we there yet? *Immunity* 1997;7:1-1.
15. Batra V, Khurana S, Musani AI, Hastie AT, Carpenter KA, Zangrilli JG, *et al.* Concentration of cytokines and growth factors in BAL fluid after allergen challenge in asthmatics and their effect on alpha-smooth muscle actin and collagen III synthesis by human lung fibroblasts. *Chest* 2003;123:398S-9S.
16. Davoodi P, Mahesh PA, Holla AD, Vijayakumar GS, Jayaraj BS, Chandrashekara S, *et al.* Serum levels of interleukin-13 and interferon-gamma from adult patients with asthma in Mysore. *Cytokine* 2012;60:431-7.
17. Patelis A, Janson C, Borres MP, Nordvall L, Alving K, Malinovschi A. Aeroallergen and food IgE sensitization and local and systemic inflammation in asthma. *Allergy* 2014;69:380-7.
18. Patelis A, Gunnbjörnsdóttir M, Malinovschi A, Matsson P, Onell A, Högman M, *et al.* Population-based study of multiplexed IgE sensitization in relation to asthma, exhaled nitric oxide, and bronchial responsiveness. *J Allergy Clin Immunol* 2012;130:397-40200.
19. Miller MR, Hankinson J, Brusasco V, Burgos F, Casaburi R, Coates A, *et al.* ATS/ERS task force: Standardisation of lungfunction testing. *Eur Respir J* 2005;26:319-38.
20. Gaur SN, Singh BP, Singh AB, Vijayan VK, Agarwal MK. Guidelines for practice of allergen immunotherapy in India. *Ind J Allergy Asth Appl Immunol.* 2009;23:1-21.
21. Nieves A, Magnan A, Boniface S, Proudhon H, Lanteaume A, Romanet S, *et al.* Phenotypes of asthma revisited upon the presence of atopy. *Respir Med* 2005;99:347-54.
22. Khilnani GC, Tiwari P. Air pollution in India and related adverse respiratory health effects: Past, present, and future directions. *Curr Opin Pulm Med* 2018;24:108-16.
23. Brandt EB, Myers JM, Ryan PH, Hershey GK. Air pollution and allergic diseases. *Curr Opin Pediatr* 2015;27:724-35.
24. Kumar R, Sharan N, Kumar M, Bisht I, Gaur SN. Pattern of skin sensitivity to various aeroallergens in patients of bronchial asthma and/or allergic rhinitis in India. *Indian J Allergy Asthma Immunol.* 2012;26:66-72.
25. Arbes SJ Jr., Gergen PJ, Vaughn B, Zeldin DC. Asthma cases attributable to atopy: Results from the Third National Health and Nutrition Examination Survey. *J Allergy Clin Immunol* 2007;120:1139-45.
26. Romagnani S. Th1 and Th2 in human diseases. *Clin Immunol Immunopathol* 1996;80:225-35.
27. Burrows B, Martinez FD, Halonen M, Barbee RA, Cline MG. Association of asthma with serum IgE levels and skin-test reactivity to allergens. *N Engl J Med* 1989;320:271-7.
28. Bousquet J, Chanez P, Lacoste JY, Barnéon G, Ghavanian N, Enander I, *et al.* Eosinophilic inflammation in asthma. *N Engl J Med* 1990;323:1033-9.
29. Robinson DS, Hamid Q, Ying S, Tsicopoulos A, Barkans J, Bentley AM, *et al.* Predominant TH2-like bronchoalveolar T-lymphocyte population in atopic asthma. *N Engl J Med* 1992;326:298-304.
30. Kips JC. Cytokines in asthma. *Eur Respir J Suppl* 2001;34:24s-33s.
31. Gupta N, Goel N, Kumar R. Correlation of exhaled nitric oxide, nasal nitric oxide and atopic status: A cross-sectional study in bronchial asthma and allergic rhinitis. *Lung India* 2014;31:342-7.
32. Kumar R, Gupta N. Exhaled nitric oxide atopy, and spirometry in asthma and rhinitis patients in India. *Adv Respir Med* 2017;85:186-92.
33. Roberts G, Golder N, Lack G. Bronchial challenges with aerosolized food in asthmatic, food-allergic children. *Allergy* 2002;57:713-7.