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Influence of Sensitization Patterns on Fractional Exhaled Nitric Oxide in Asthmatic Children

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ABSTRACT

Fractional exhaled nitric oxide (FeNO) has been suggested as a non-invasive biomarker of airway inflammation, which is increased in atopic subjects. Whether sensitization to particular allergens is a predictive factor for increased FeNO levels is not yet fully understood.

We conducted a retrospective cross-sectional study. From October to December in 2015, the medical documents of 127 mild, steroid-naive asthmatic children and 34 healthy age-matched children were enrolled in this study. The results of the FeNO measurements, skin prick test, and the spirometry were collected for analysis.

Sensitization patterns to the 18 aeroallergens (5 categories: mites, molds, animal dander, pollen, and other) were determined in study population. A significant increase in FeNO level was observed in poly-sensitized asthmatic children (34.7 part per billion, (ppb) [28.3-41.1 p.p.b]), compared with mono-sensitized asthmatics (30.7 p.p.b [18.3-43.2 p.p.b]) and with non-sensitized asthmatics (17.3 p.p.b [10.8-24.5 p.p.b]). With sensitization to perennial allergens (mites, mold, and animal dander), blood eosinophil counts were significantly associated with increased FeNO ($p<0.05$ for all). The highest FeNO level was identified in children sensitized to a combination of the perennial, seasonal, and other allergens, when compared with those sensitized to one category of allergen alone ($p=0.004$).

Our study showed that variations in FeNO level were associated with individuals' sensitization patterns. Being sensitized to some particular allergens might contribute to prompt the airway inflammation.

Keywords: Asthma; Atopy; Exhaled nitric oxide; Perennial allergens; Sensitization patterns; Skin prick test

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INTRODUCTION

Asthma, a heterogeneous group of disorders, is characterized by chronic airway inflammation with

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airway hyper-responsiveness, reversible airflow obstruction, and airway remodeling.[1] In clinical practice, the level of asthma control is usually evaluated according to the clinical symptoms, usage of rescue medication, and inflammation status.[2,3] Although the assessments of airway inflammation could be determined in the bronchial biopsy, bronchoalverlar lavage, or induced sputum, the above tests are limited in pediatric practice due to their traumatic and costly nature.[4,5]

Fractional exhaled nitric oxide level (FeNO), a biomarker for eosinophil mediated airway inflammation, has been proven to be higher in atopic asthmatics than in non-atopic asthmatics.[6-8] Atopy is defined as being sensitized to any allergens, with the presence of serum IgE or positive skin prick testing (SPT) reactions. The latest studies show that there are multiple "sensitization patterns" that may be linked to a much greater probability of developing poorer lung function and severer airway reactivity, which is decided by the specific features of allergen protein.[9,10] On the basis of the above finding that sensitization to particular allergens might play a role in asthma development, it is plausible that sensitization to different types of allergens is associated with airway inflammation based on FeNO level accordingly. Meanwhile, in China it was reported that up to 72.1% children suffering from asthma and/or rhinitis were sensitized.[11]Given high sensitization prevalence existing in Chinese children, we sought to verify whether sensitization to a specific allergen might have an influence on FeNO levels over other allergens in a sample of Chinese asthmatic children.

MATERIALS AND METHODS

Study Design

We conducted a retrospective, cross-sectional study with the goal of assessing the differences in FeNO levels among asthmatic children with various sensitization patterns. We collected the medical documents of 127 mild to moderate asthmatic children who attended our Allergic Outpatient Clinic from October to December in 2015. The definition and severity of asthma was based on the Global Initiative for Asthma guidelines. The following results, including FeNO measurements, skin prick tests, and spirometry, were collected to analyze. In addition, another 34 healthy children who completed a health examination in the Healthcare Center were invited to join in this study as the control group.

All the participants were chosen from Northern China and were naïve to control treatment for 1 month or more: our aim was to avoid the fluctuating nature of FeNO concentrations that varied with the change in natural exposure to environment allergen, and to minimize the influence on the FeNO values induced by the steroid usage. The Beijing Children's Hospital institutional review board approved the entire study protocol (No. 2014-104), and written informed consent was obtained from all participants.

FeNO Measurement

In our allergic outpatient clinic, FeNO was measured prior to the spirometry on the same day. Fractional exhaled nitric oxide was measured online using an Aerocrine NIOX chemiluminescenceanalyzer (Solna, Sweden) at a flow rate of 50 mL/s. The lower and upper limits of detection were 2 and 200 part per billion (p.p.b), respectively. The device was operated and calibrated in accordance with the manufacturer's instructions, and FENO was measured according to American Thoracic Society/European Respiratory Society (ATS/ERS) guidelines.[13]

Allergen Sensitization

Sensitization patterns to common aeroallergens were determined by SPT, and the following 18 allergens in 5 categories were measured:

1) Mites: *Dermatophagoidespteronyssinus*, *Dermatophagoidesfarinae;* 2) Molds: *Penicilliumchrysogenum*, *Cladosporiumcladosporioides*, *Alternariaalternata*, *Aspergillusfumigatus;* 3) Animal dander: cat dander, dog dander; 4) Pollen: *Artemisia sieversiana*, *Humulusscandens*, *Ambrosia artemisifolia*, *Chenopodium album*, *Sabina chinensis*, *Fraxinus Americana*, *Ailanthus altissima*, *Platanusacerifolia*, *Betulaplatyphylla*; 5) Other: *German cockroach*. A positive control (histamine 10 mg/mL) and negative control (saline) were also included in each test. A drop of the allergen was placed onto the skin surface in the volar surfaces of both forearms, and vertically introduced into the epidermis using individual stainless steel lancets. Excess allergen was often removed with gauze or tissue paper. Any immediate reaction (wheal or erythema) was read 20 minutes later and recorded with a fine-tip pen. The wheals were transferred to a permanent paper record using sticky tape. The wheal

size was recorded in millimeters as the long axis and its perpendicular; the mean of these 2 measurements was calculated. A positive reaction was defined as a mean wheal diameter equal or greater to that in positive group. Based on the numbers of positive allergens, subjects being sensitized to only one category of allergens were considered as the mono-sensitized, while those sensitized to two or more categories of allergens were considered to be the poly-sensitized.

Pulmonary Function Testing

Spirometry was performed using a computerized spirometry (Jaeger-Toennies GmbH, Hoechberg, Germany) in accordance with the recommendations of the American Thoracic Society.

Statistical Analysis

Statistical analyses were performed using SPSS 19.0 software (SPSS Inc., Chicago, IL, USA). The FeNO values and blood eosinophil counts were log transformed to obtain the approximately normal distribution. Data were presented as mean±SD or geometric mean (range of 1 SD). The variable was compared between different groups using one-way analysis of variance (ANOVA) or the chi-square test for multiple comparisons, as appropriate. The interrelationship among the FeNO level, blood eosinophil counts, and individual's sensitization pattern was detected using a linear regression model, with adjustment of age, gender, and body mass index. A p value less than 0.05 was considered to be statistically significant.

RESULTS

Data obtained from 127 mild, steroid-naive asthmatic children and 34 healthy subjects were included in present study. The baseline clinical characteristics of the study population are shown in Table 1. First, it was found that obviously higher FeNO levels occurred in asthmatic children in comparison with healthy children (26.7p.p.b [7.3-41.1p.p.b] vs 7.9p.p.b [4.5-13.6 p.p.b]; $p<0.001$). Among healthy subjects, the FeNO levels between children with or without sensitization state were comparable, and the difference was non-significant (non-sensitized healthy subjects: 6.9 p.p.b (5.5-9.5) vs. Sensitized Healthy subjects: 8.3 p.p.b (7.6-13.6); $p>0.05$). Among enrolled asthmatic children, 98 (77.2%) were atopic, of which 39 (30.7%) were the mono-sensitized, and the remaining 59 (46.5%) were the poly-sensitized. FeNO levels increased significantly with the higher number of sensitized allergens (non-sensitized: 17.3p.p.b [10.8-24.5p.p.b]; mono-sensitized: 30.7p.p.b [18.3-43.2p.p.b]; poly-sensitized: 34.7p.p.b [28.3-41.1p.p.b]; $p<0.001$). A further inter-group

Table 1. Demographic and clinical characteristics of asthmatic children with different sensitization patterns and healthy subjects

	Non-sensitized healthy subjects (n=10)	Sensitized healthy subjects (n=24)	Non-Sensitized asthmatics (n=29)	Mono-sensitized asthmatics (n=39)	Poly-sensitized asthmatics (n=59)
Age mean	13.2	11.6	8.7	7.9	9.6
(range)	(8.6-14.6)	(9.2-14.0)	(4.0-12.4)	(5.2-9.4)	(6.8-13.4)
Gender (boy/girl)	5/5	15/9	10/19	12/27	46/13
BMI (Kg/m^2)†	19.8±3.4	17.8±6.9	15.1±2.9	15.1±3.5	17.2±2.9
FEV1 (%Pred)†	99.8±15.8	96.2±13.7	91.2±11.9	90.8±11.5	89.0±28.8
FVC (% Pred)†	96.3±19.4	95.7±15.3	93.4±12.3	91.4±11.3	91.4±14.2
Mean wheal size (mm)	-	4.5±1.6	-	3.7±1.9	4.0±3.6
EOS (/ul)*	78.6	75.3	183.3	333.6	436.5
	(43.2-110.5)	(67.2-100.1)	(70.9-241.85)	(141.0-525.7)	(131.4-1013)
FeNO (p.p.b)*	6.9	8.3	17.3	30.7	34.7
	(5.5-9.5)	(7.6-13.6)	(10.8-24.5)	(18.3-43.2)	(28.3-41.1)

BMI, body mass index; FeNO, factional exhaled nitric oxide; FEV1, forced expiratory volume in 1 second; FVC, forced vital capacity; EOS, blood eosinophil; p.p.b, parts per billion† Mean±SD;

*Geometric mean (range of 1 SD)

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comparison was performed between the mono-sensitized group and the poly-sensitized group, which indicated that a higher FeNO level appeared in the poly-sensitized group than in the mono-sensitized group, with a significant difference (p<0.001, Figure 1).

To explore interaction between sensitization patterns and FeNO levels, it was revealed that being sensitized to mites (p=0.004), molds (p=0.014), and animal dander (p=0.09) was more likely associated with the elevated FeNO levels (Table 2). Using a linear regression model, it was shown that blood eosinophil counts and sensitization to the perennial allergens were significant predictors for increased FeNO in asthmatic children. Moreover, a highest FeNO level was identified in children sensitized to a combination of the perennial, seasonal, and other allergens, when compared with those sensitized to one category of allergen alone (p=0.004) (Table 3).

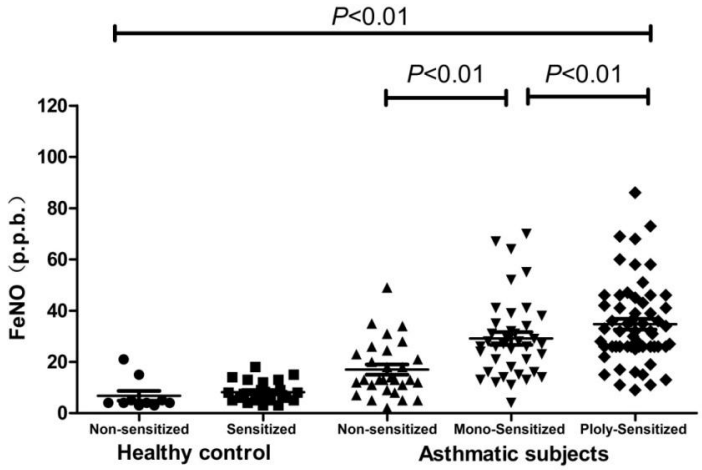

Figure 1. Distribution of fractional exhaled nitric oxide (FeNO) levels among healthy subjects and asthmatic subjects
Box-plot explanation: upper horizontal line of box, 75th percentile; lower horizontal line of box, 25th percentile; horizontal bar within box, median; upper horizontal bar outside box, upper adjacent value; lower horizontal bar outside box, lower adjacent value.

Table 2. Fractional exhaled nitric oxide level (FeNO) levels measured in relation to sensitization patterns in asthmatic children

Category of allergen	Number of the sensitized subjects (%)	FeNO* (p.p.b)		*p* value
		Sensitized		
		Yes	No	
Mites **(***Dermatophagoidespteronyssinus, Dermatophagoidesfarinae***)**	72 (56.7)	38.19 (18.2-59.2)	25.6 (14.8-35.1)	**0.004**
Molds **(***Penicilliumchrysogenum, Cladosporiumcladosporioides, Alternariaalternata,Aspergillusfumigatus)*	40 (31.5)	35.4 (11.2-56.9)	26 (16.5-38.7)	**0.014**
Animal dander (cat dander, dog dander)	37 (29.1)	29.7 (22.4-36.1)	25.3 (12.4-39.4)	**0.09**
Pollens (grass, tree, weed)	16 (12.6)	25.2 (23.6-27.2)	24.8 (21.4-28.1)	0.45
Other (German cockroach)	8 (6.3)	31.6 (11.5-53.8)	29.5 (15.2-36.2)	0.309

*Geometric mean (range of 1 SD)

Table 3. Multivariate linear regression of factors associated with fractional exhaled nitric oxide concentration (FeNO) value in asthmatic children (N=127)

Variable	FeNO β (95% CI)†	*p* value
EOS (/uL)*	0.15 (0.3-0.31)	**0.032**
Mean wheal size (mm)	0.23(0.16-0.37)	0.358
Number of positive skin testing	0.29(0.14-0.64)	0.427
Sensitized to		
Perennial allergens only	0.42(0.17-0.41)	**0.012**
Seasonal allergens only	0.28(0.12-0.46)	0.658
Other allergen only	0.13(0.11-0.34)	0.924
Perennial and seasonal allergens	0.36(0.13-0.64)	**0.024**
Seasonal allergens and other allergens	0.22(0.14-0.51)	0.523
Perennial allergens and other allergens	0.27(0.06-0.61)	**0.034**
Perennial, seasonal and other allergens	0.57(0.17-0.73)	**0.004**

EOS, blood eosinophil; Perennial allergens, Mites, molds, and animal dander Seasonal allergens, grass, weed, and tree pollens; Other allergen: German cockroach

†FeNO levels are presented as geometric mean (range of 1 SD)

*Adjusted covariates include age, gender, and BMI.

DISCUSSION

In this study, we attempted to demonstrate the interrelationship between the individuals' sensitization patterns and FeNO levels in a sample of Chinese asthmatic children. Although association relationship between atopy and FeNO levels had been conducted before, the difference in FeNO levels induced by sensitization to particular allergens has not been documented clearly, especially in asthmatic children.

One of the main findings of our study was that the FeNO level is higher in asthmatic children than in the healthy children, which reconfirmed the earlier findings showing FeNO levels as a biological reflection marker for airway inflammation. We also found that the FeNO levels were positively related to the sensitization degree, which was defined as the number of positive skin responses, and that a higher FeNO level was more likely to occur in the poly-sensitization asthmatic group compared to the mono-sensitization group. Our result was in line with those of Jang WN et al. showing that the sensitization degree, defined as the sum of the specific IgE, may have a dose-response effect on FeNO levels in Korean asthmatic children.[14] Although the mechanism by which an increase in the degree of atopic responsiveness could induce a rise in FeNO is not fully clear, it has been proven that the numbers of interlukin-4 (IL-4) positive T lymphocytes cells in the bronchial biopsies from the atopic asthmatics were significantly higher compared with those in the non-atopic asthmatics. IL-4 was considered to be the most powerful cytokine to induce FeNO production in the asthmatic airway.[15] Moreover, it is evident that an increasing degree of atopic responsiveness could trigger the downstream signal and upregulated inducible nitric oxide synthase (iNOS) mRNA expression, eventually forming a positive-feedback loop among "allergen sensitization, iNOS production, and FeNO elevation".[16-18]

Furthermore, whether FeNO level was attributable to some particular allergens was another focus of this study. We found that sensitization to perennial allergens like mites, molds, and animal dander was significantly associated with increased FeNO values. Our results are consistent with previous studies showing that sensitization to certain groups of perennial allergens like pet dander and house dust mite (HDM) would possess a much higher FeNO level over other groups of allergens.[19-21] Ekrooshad reported that FeNO level was significantly higher in patients who were both sensitized and exposed to indoor allergens in comparison with those who were sensitized but not exposed.[22] This suggested that the FeNO level should be considered as a marker of airway inflammation

induced by domestic allergens in sensitized asthmatics. Furthermore, Simpson et al. compared the difference in FeNO levels of atopic subjects with exposure to different levels of HDM [Dermatophagoides P1 (*Der*P1)], cat dander [Felisdomesticus allergen 1, (*Fel*d1)], and dog dander [Canis familiaris allergen 1, (*Can* f1)] allergens. It was shown that when compared with other indoor allergens, being sensitized and exposed to mites was the main determinant for increased FeNO concentrations in sensitized subjects, owing to the universal and high level of existence of HDM.[23] On the basis of the findings listed above, it is believed that the relationship between FeNO and allergic sensitization is not only qualitative but also quantitative, and the allergens exposure level would modify this relationship. In present study, the FeNO measurements were detected in winter, when the concentration of seasonal allergens was expected to be the lowest throughout the whole year. Beyond that, in winter people would have more time to stay home; therefore, being exposed to a relative higher level of indoor allergens than other seasons, which eventually led to a higher FeNO level. We assumed the difference in the type and level of allergen exposure might attribute to why we were unable to establish the relationship between FeNO levels and season allergens (i.e., pollens), as reported from previous studies conducted in other countries.[24-25]

There are some limitations to this study. First, our study design is retrospective, which did not allow us to measure allergen levels at home and detect possible dose-effect relationship among allergen exposure and airway inflammation status based on the FeNO level. Moreover, the clinical data were obtained from the patients' medical documents, which could have been partly influenced by the selection bias. Second, the sample size was small. Because we only recruited children from Northern China, these results are not representative of all Chinese children. It should be noted that the extrapolation of the results to children living in other areas needs further confirmation. Finally, only inhalant allergens were examined in this study; the effects of sensitization to food on FeNO levels should be considered in future.

In summary, FeNO was considered as biomarker to reflect the airway inflammation in asthmatic children. Our study provided the evidence that variations in the FeNO level were associated with the individual's sensitization patterns, and being sensitized to some particular allergens might contribute to prompt the FeNO production. Therefore, it is rational to consider the influences caused by individual's sensitization patterns to gain a correct and objective interpretation of FeNO values.

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