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ORIGINAL ARTICLE

Exhaled nitric oxide as a better diagnostic indicator for evaluating wheeze and airway hyperresponsiveness in preschool children

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Abstract

Objective: Fractional concentration of exhaled nitric oxide (FeNO) is a known marker of airway inflammation. The aims of this study were to evaluate FeNO, impulse oscillometry (IOS), and spirometry in preschool children and to investigate their relationship with wheeze and airway hyperresponsiveness (AHR). **Methods:** We performed a population-based, cross-sectional study with 561 children aged 5–6 years. A total of 544 children completed a modified International Study of Asthma and Allergies in Childhood (ISAAC) questionnaire and eligible for the study. We measured FeNO, spirometry, methacholine bronchial provocation, and IOS. AHR was defined as the induction of a 20% decrease in FEV₁(PC₂₀) by a methacholine concentration ≤8.0 mg/dL. **Results:** Children who had wheeze or AHR had higher FeNO levels than children without these symptoms. However, neither IOS nor spirometry parameters showed significant differences between children with wheeze or AHR and those without. FeNO was associated with AHR, whereas IOS or spirometry parameters showed no association. Mean FeNO levels were positively correlated with a dose–response slope for methacholine, but neither IOS nor spirometry parameters showed significant correlations. **Conclusions:** FeNO is a more sensitive measurement of AHR and wheeze than spirometry or IOS in preschool children.

Keywords

Airway hyperresponsiveness, airway inflammation, fractional exhaled nitric oxide, impulse oscillometry, lung function, preschool children

History

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Introduction

Airway inflammation is a common component of the pathophysiology of asthma and is linked to airway hyperresponsiveness (AHR) and airway obstruction [1]. Airway inflammation is present in mildly asthmatic patients who have normal lung function and even in patients with well-controlled asthma [2].

Persistence of airway inflammation is known to be a leading cause of recurrence of asthma and airway remodeling.

Spirometric analysis to assess airway obstruction is the most commonly used test in asthma, but requires effort from the patient and the results are usually normal in cases of mild asthma. Impulse oscillometry (IOS) to assess airway resistance is effort-independent, but not yet standardized for the diagnosis of bronchial asthma [3]. A bronchial provocation test to assess AHR can discriminate mild asthma from asthma mimetic conditions [4]. However, measurement of AHR by bronchial provocation test is time consuming and requires experienced technicians and great effort from the patient, therefore its use in preschool children has some limitations. As a result, preschool children with asthma are usually diagnosed based purely on clinical symptoms because of their limited ability to follow directions or give maximal and reproducible efforts.

The fractional concentration of exhaled nitric oxide (FeNO) is a non-invasive and simple measurement of

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airway inflammation [5,6] that can be performed even in preschool children. Although studies on the relationship of FeNO to other lung function measurements in asthma have been reported for school children [7], there is a scarcity of data for preschool children. Furthermore, there is no clear conclusion on the correlation between FeNO and other lung function measurements. Some studies have shown superiority of FeNO over spirometry or bronchodilation test in AHR or asthma [8,9], whereas others reported a lower sensitivity of FeNO in the diagnosis of asthma [10]. Furthermore, there has been no study comparing FeNO, bronchodilator response (BDR) by IOS, and spirometric parameters in preschool children.

In this study, we assessed FeNO levels as well as parameters of spirometry and IOS, and compared them in a population-based study of preschool children to investigate their relationship with wheeze and AHR.

Methods

Study population

We conducted a cross-sectional, community-based study at 16 preschools in Seoul and the Gyeonggi province of Korea. This study was performed as a government-funded program to determine a standardized measurement of lung function and the prevalence of allergic diseases in preschool children.

A total of 561 children aged 5–6 years were invited to participate and 551 participants completed a modified International Study of Asthma and Allergies in Childhood (ISAAC) questionnaire. The questionnaire was based on the Korean version of ISAAC [11]. After exclusion of children who were taking medications due to respiratory tract infections or asthma during the last 14 d, 544 preschool children were eligible for the study. A skin prick test was performed in 446 children, and measurements were performed for FeNO ($n = 343$), spirometry ($n = 433$), IOS ($n = 356$), and methacholine bronchial provocation ($n = 211$) (Figure 1). All the tests were administered by the same well-trained researchers to ensure standardization of the survey results.

The study protocol was approved by the institutional review boards of the University of Ulsan College of Medicine. Written informed consent was obtained from parents or guardians of all participants after they had been fully informed of the details of the study.

FeNO measurement

FeNO levels were measured with a portable electrochemical device (NIOX MINO; Aerocrine AB, Stockholm, Sweden) as we described in a previous study [11]. Briefly, children inhaled through a nitric oxide scrubbing filter and exhaled into the room air twice. After a third inhalation through the filter, the children exhaled into the analyzer for at least 6 s to ensure a stable NO level. Exhaled FeNO was measured at a flow rate of 50 mL/s without a nose clip. The procedure was repeated three times for each subject and the median value was used for data analysis. We performed the FeNO measurements first, followed by spirometry, IOS, and the methacholine provocation test, because a spirometry test prior to FeNO measurement can reduce FeNO levels.

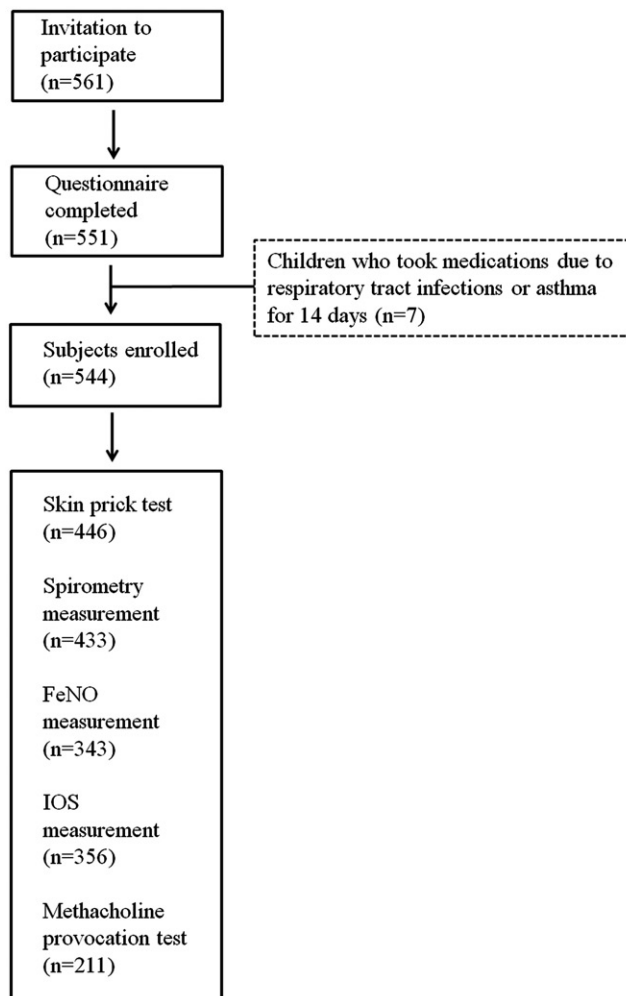


Figure 1. Schematic presentation of recruitment of study subjects.

Spirometry and methacholine bronchial provocation test

A pulmonary function test was performed using a Vmax 22 (Sensormedics, Yorba Linda, CA) following ATS/ERS guidelines [12]. Forced expiratory volume in 1 s (FEV₁) and forced vital capacity (FVC) were measured by a standard spirometric method, and the best of three forced expiratory volume curves was used to determine the FVC and FEV₁ values.

The methacholine bronchial provocation test was conducted as previously described [11]. Airway responsiveness is expressed as the provocative concentration of methacholine required to induce a 20% decrease in FEV₁ (PC₂₀), and for this study AHR was defined as a PC₂₀ value ≤ 8 mg/mL.

The dose–response slope (DRS) was defined as % decline of FEV₁ divided by the final cumulative dose of methacholine administered.

Impulse oscillometry (IOS)

Impulse oscillometry was performed using the Master Screen Spirometry-IOS Digital System (Jaeger Company, Würzburg, Germany). IOS measurements were taken as previously published [11,13], following ATS/ERS guidelines [12]. Children breathed normally into a mouthpiece for a short period of time, during which a loudspeaker delivered

a pulse-shaped pressure flow excitation to the respiratory system. Rrs and Xrs at 5, 10, 15, 20, 25, and 35 Hz, total Zrs, and the resonance frequency (Fr, the frequency at which reactance equals zero) were recorded. The bronchodilator response (BDR) was assessed by repeating the measurements 15 min after inhalation of 200 µg of salbutamol and was calculated as relative change (percent change from baseline). A positive BDR was defined as a greater than 20% increase in R5Hz after bronchodilator inhalation [12]. All study procedures were conducted by trained researchers under the supervision of pediatric allergy specialists.

Allergic sensitization

A skin prick test (Allergopharma, Reinbek, Germany) was performed using 16 common allergens: house dust mites (*Dermatophagoides pteronyssinus* and *Dermatophagoides farinae*), animal dander (cat and dog epithelia), pollen (mugwort, ragweed, grass, and tree pollen mix 1 and 2), molds (*Aspergillus fumigatus* and *Alternaria alternata*), cockroaches (*Blattella germanica*), and food (milk, soybean, egg white, and peanut). Histamine was used as a positive control and isotonic saline was used as a negative control. The skin prick test was considered positive when the mean wheal size in response to an allergen was ≥ 3 mm and greater than or equal to the mean wheal size in response to histamine. Atopy was defined as a positive skin prick test for any allergen.

Measurement of serum total IgE and eosinophils

A venous blood sample was obtained from children for whom we had received a complete questionnaire and parental consent. Serum total IgE concentrations were measured by fluorescent enzyme immunoassay (Pharmacia CAP System; Pharmacia Diagnostics AB, Uppsala, Sweden). Blood eosinophils were counted using an automatic blood cell counter (XE-100, Sysmex Co., Kobe, Japan).

Statistical analysis

Statistical analyses were performed using STATA version 11.0 (StataCorp LP, College Station, TX). Mean values were compared between two different groups using Student's *t*-test and among three groups using one-way ANOVA. The Bonferroni *post hoc* test was used for multiple comparisons. The significance of differences in categorical variables between groups was tested using Chi-square analysis. Multivariate logistic regression was used to determine the associations of lung function tests with AHR. Adjusted odds ratios (aORs) and 95% confidence intervals (CIs) were derived after adjusting for age, sex, height, and weight. All data were expressed as means $\pm$ SDs and significance was defined as a *p*-value less than 0.05.

Results

Clinical characteristics of study subjects

The mean age of study participants was 5.5 years and 51.7% were male. The prevalence of allergic diseases was determined based on responses to the ISAAC questionnaire as follows: any history of wheezing, 23.5%; asthma, 10.1%;

allergic rhinitis, 27.9%; and atopic dermatitis, 35.4%. The prevalence of AHR, positive bronchodilator response by IOS, atopic sensitization, and environmental tobacco smoke (ETS) exposure was 34.6%, 20.0%, 23.1%, and 46.8%, respectively. The ETS exposure was defined as being exposed to ETS at least one time per week. There were no differences in FeNO, spirometry, IOS parameters, and DRS between children with ETS exposure and those without exposure (data not shown).

Comparison of FeNO, spirometry, and IOS according to wheezing

We compared FeNO levels, spirometric and IOS parameters, DRS by methacholine bronchial provocation test, and bronchodilator response by IOS between children who ever had wheeze and those without wheeze (Table 1).

There were no differences in age, sex, weight, or height between the two groups. FeNO levels were significantly increased in children with wheeze compared to those without, whereas there was no difference in DRS by methacholine bronchial provocation test, parameters of spirometry or IOS, and the prevalence of positive BDR by IOS. Blood eosinophil levels were higher in the group with wheeze ever than in the group without. However, serum total IgE levels and the prevalence of atopy did not differ significantly between the two groups.

Comparison of FeNO, spirometry, and IOS between children with and without AHR

Since AHR is one of the most characteristic findings in asthma, we compared levels of FeNO, spirometric and IOS parameters, DRS by methacholine bronchial provocation test, and bronchodilator response by IOS between children with AHR and those without (Table 2).

There were no significant differences in age, height, or body weight between the two groups. There was no gender

Table 1. Comparison of FeNO, spirometry, and IOS according to wheeze.

	Wheeze ever (–) (n = 416)	Wheeze ever (+) (n = 128)	<i>p</i> Value
Age (years)	5.8 $\pm$ 0.4	5.8 $\pm$ 0.5	0.793
Male (%)	49.1	52.7	0.558
Weight (kg)	20.8 $\pm$ 3.5	21.4 $\pm$ 3.4	0.178
Height (cm)	114.0 $\pm$ 5.4	115.9 $\pm$ 6.6	0.052
FeNO (ppb)	10.01 $\pm$ 5.86	13.61 $\pm$ 8.48	0.003
DRS	1.77 $\pm$ 0.56	1.83 $\pm$ 0.63	0.568
FEV ₁ (% predicted)	96.9 $\pm$ 15.1	96.6 $\pm$ 12.7	0.882
FEV ₁ /FVC (%)	94.5 $\pm$ 5.8	94.6 $\pm$ 4.6	0.860
FEF _{25–75} (% predicted)	96.2 $\pm$ 24.9	94.5 $\pm$ 20.5	0.653
R5Hz (% predicted)	128.1 $\pm$ 24.8	129.6 $\pm$ 35.8	0.720
X5Hz (% predicted)	107.2 $\pm$ 37.6	117.4 $\pm$ 49.7	0.145
IOS BDR (%)	19.4	25.4	0.364
Eosinophil (%)	4.0 $\pm$ 3.0	5.4 $\pm$ 4.4	0.017
LogIgE (IU/mL)	1.88 $\pm$ 0.53	1.95 $\pm$ 0.58	0.332
Atopy (%)	23.7	35.2	0.067

DRS, dose–response slope; FEV₁, forced expiratory volume in 1 s; FVC, forced vital capacity; FEF_{25–75}, mid forced expiratory flow; R5Hz, resistance at 5 Hz; X5Hz, reactance at 5 Hz; IOS BDR, impulse oscillometry bronchodilator response; logIgE, logarithmic transformation of IgE.

association for wheeze, but the prevalence of AHR was higher in females.

FeNO levels in children with AHR were significantly higher than those in children without AHR. There were no differences in spirometry between the two groups, although FEV₁ showed a tendency to be lower in the AHR group ($p = 0.06$). There were no differences in R5Hz, X5Hz, or the prevalence of positive BDR by IOS between the two groups. Levels of blood eosinophils and serum total IgE were significantly increased in children with AHR compared to those without. The prevalence of atopy tended to be higher in children with AHR than in those without AHR ($p = 0.054$).

Association of FeNO and other lung function parameters with AHR

We investigated the association of FeNO, spirometry, and IOS parameters with AHR. FeNO was significantly associated with AHR when adjusted for age, sex, height, and weight

Table 2. Comparison of FeNO, spirometry, and IOS according to AHR.

	AHR (–) (<i>n</i> = 138)	AHR (+) (<i>n</i> = 73)	<i>p</i> Value
Age (year, mean ± SD)	6.1 ± 0.4	6.1 ± 0.4	0.927
Male (%)	49.3	31.5	0.010
Height (cm)	116.6 ± 5.7	115.9 ± 4.4	0.363
Weight (kg)	21.9 ± 3.8	21.8 ± 3.4	0.553
FeNO (ppb)	9.93 ± 5.36	14.08 ± 9.81	0.003
DRS	1.46 ± 0.37	2.40 ± 0.36	0.001
FEV ₁ (% predicted)	99.0 ± 14.9	95.5 ± 10.7	0.064
FEV ₁ /FVC (%)	94.6 ± 6.1	95.0 ± 4.0	0.680
FEF _{25–75} (% predicted)	99.2 ± 26.3	98.5 ± 19.9	0.781
R5Hz (% predicted)	129.8 ± 23.9	128.7 ± 23.9	0.797
X5Hz (% predicted)	113.6 ± 40.0	109.5 ± 44.5	0.565
IOS BDR (%)	18.0	25.0	0.385
Eosinophil (%)	3.7 ± 2.7	5.0 ± 3.7	0.016
logIgE (IU/mL)	1.88 ± 0.52	2.12 ± 0.63	0.007
Atopy (%)	22.2	36.9	0.054

AHR, airway hyperreactivity; DRS, dose–response slope; FEV₁, forced expiratory volume in 1 s; FVC, forced vital capacity; FEF_{25–75}, mid forced expiratory flow; R5Hz, resistance at 5 Hz; X5Hz, reactance at 5 Hz; IOS BDR, impulse oscillometry bronchodilator response; logIgE, logarithmic transformation of IgE.

(aOR, 1.078; 95% CI, 1.027–1.131). However, parameters of spirometry, IOS and bronchodilator response by FEV₁, and R5Hz revealed no significant associations with AHR (Table 3).

Correlation of FeNO with DRS and parameters of spirometry and IOS

Mean FeNO levels positively correlated with DRS ($r = 0.228$, $p = 0.004$), but not with FEV₁ ($r = -0.075$, $p = 0.162$), R5Hz ($r = -0.053$, $p = 0.386$), or X5Hz ($r = 0.048$, $p = 0.425$) (data now shown).

Discussion

In this study, we measured FeNO and performed other lung function tests, such as spirometry, IOS, and methacholine bronchial provocation, and investigated the data according to presence or absence of wheeze or AHR in preschool children from 16 preschools in Seoul and the Gyeonggi province of Korea. There have been some studies comparing FeNO with spirometry or IOS based on clinical diagnosis of asthma in preschool children [14], but to the best of our knowledge, there is no published study in which FeNO levels have been compared with spirometry and IOS according to AHR in preschool children.

Children in this study with wheeze or AHR showed higher levels of FeNO. However, other lung function parameters assessed with spirometry or IOS showed no differences between children with wheeze or AHR and those without. Airway obstruction, airflow variability, and airway inflammation are the main features of asthma. However, patients with mild asthma often show no airflow limitation or variability. Airway inflammation assessed by FeNO demonstrated high sensitivity and specificity to identify AHR in adult patients with asthma-like symptoms but normal spirometry or negative BDR [8]. Lieven et al. not only found that exhaled NO levels were higher in patients with AHR but also found a significant correlation between exhaled NO levels and the degree of AHR in steroid-naïve patients [15].

In this study, neither IOS and spirometric parameters nor BDR showed significant associations with AHR. An increase

Table 3. Association of FeNO and other lung function parameters with AHR.

	AHR		
	Crude OR	Model 1 ^a	Model 2 ^b
FeNO (ppb)	1.079 (1.030–1.132)	1.075 (1.026–1.126)	1.078 (1.027–1.131)
FEV ₁ (% predicted)	0.981 (0.960–1.003)	0.987 (0.964–1.010)	0.981 (0.956–1.006)
FEV ₁ /FVC (%)	1.013 (0.946–1.083)	1.015 (0.947–1.088)	1.012 (0.943–1.087)
FEF _{25–75} (% predicted)	0.998 (0.983–1.013)	0.999 (0.984–1.015)	1.0001 (0.984–1.017)
R5Hz (% predicted)	0.998 (0.984–1.012)	0.999 (0.984–1.014)	0.997 (0.982–1.013)
X5Hz (% predicted)	0.998 (0.989–1.005)	0.997 (0.989–1.006)	0.997 (0.988–1.006)
Bronchodilator response			
Δ R5Hz (%)	0.966 (0.931–1.003)	0.964 (0.927–1.002)	0.964 (0.927–1.003)
Δ FEV ₁ (%)	1.030 (0.995–1.065)	1.029 (0.994–1.066)	1.031 (0.995–1.069)

Data show odds ratio [OR] (95% confidence interval) by multivariate logistic regression.
AHR, airway hyperreactivity; FEV₁, forced expiratory volume in 1 s; FVC, forced vital capacity; FEF_{25–75}, mid forced expiratory flow; R5Hz, resistance at 5 Hz; X5Hz, reactance at 5 Hz.
^aAdjusted for sex and age.
^bAdjusted for sex, age, weight, and height.

of FEV₁ by more than 12% after inhalation of bronchodilator, or a positive BDR by spirometry is considered the gold standard in the diagnosis of asthma. However, because it is difficult for young children to perform spirometry, which requires a forced maximal inhalation and exhalation, various cut-off values of FEV₁ have been suggested for preschool children [16,17]. IOS is useful in young children because it requires tidal breathing. Although there are several studies on cut-off values for IOS-assessed BDR in preschool children with asthma [18–20], there is no consensus regarding definite cut-off levels for the diagnosis of asthma. In this study, we defined a 12% change in FEV₁ and a 20% change in R5 as a positive BDR. Although FeNO levels were significantly associated with AHR, the parameters of spirometry or IOS, bronchodilator responses by FEV₁ or R5Hz, did not show associations with AHR. This suggests that FeNO is a more sensitive marker for AHR than BDR by IOS or spirometry in preschool children.

Our findings that FeNO did not correlate with any of the spirometric or IOS parameters in preschool children but was significantly correlated with AHR are supported by other studies that did not observe any correlation between elevated FeNO and impairment of spirometry parameters [21,22]. Based on our study, we assume that airway inflammation is closely linked to AHR and wheeze, but not to airflow limitation or reversibility.

FeNO is potentially a more sensitive test for asthma than spirometry [23–25] because the vast majority of children with mild asthma have normal lung function [26], whereas airway inflammation is present in asthmatic patients whose lung function is normal or clinically well controlled. This suggests that lung function tests may not be sensitive enough to reflect the extent of airway inflammation or AHR [6]. The persistence of airway inflammation can induce acute exacerbation of asthma. Therefore, careful monitoring of FeNO may enable strategies to reduce asthma exacerbation.

In this study, the percentage of preschool girls with AHR was higher than the percentage for boys. However, when we compare DRS between males and females, there was no significant difference between the two sexes. This is in agreement with other results which showed no difference in DRS between boys and girls at the age of 6 years [27]. This may be due to the cutoff point of methacholine for AHR. In this study, we defined AHR as an 8 mg/ml of methacholine dose. Until now, there has been no consensus on definite cutoff values of methacholine for AHR in preschool children under 6 years old. Age, height, weight, sex, and race can influence AHR. As children are younger and smaller, they can receive a higher dose of methacholine relative to their lung size [28].

Various factors are known to affect FeNO levels. FeNO levels increase with increased height [29] and are highest in summer and fall [30]. FeNO can also be affected by steroid therapy or smoking [15]. Therefore, when interpreting FeNO measurements we should consider the patient's age, height, the season, and any prescribed medications as possible confounding factors. For these reasons, we excluded subjects who were taking any medications due to respiratory tract infections or asthma, and adjusted for age, sex, weight, and height in multivariate logistic regression analysis.

The resulting aORs indicated that only FeNO was significantly associated with AHR.

The major strength of this study is we enrolled preschool children from the general population and assessed results from spirometry, IOS, FeNO, and methacholine bronchial provocation tests. Asthma is a heterogeneous inflammatory disorder of small airways. The diagnosis of asthma in young children can be easily over- or underestimated when it is dependent on clinical symptoms only; this can be one of the reasons for sampling bias. In this study, we tried to stratify study subjects with AHR based on objective parameters. Stratification by objective parameters can reduce bias and adds strength to our study.

Nonetheless, the present study does have some limitations. First, because this was a population-based cross-sectional study, most of the study subjects showed normal lung function. Second, because this study was conducted using preschool children aged 5–6 years, the entire set of laboratory tests was not successfully performed for every child and the sample size for each test was not large. However, it is meaningful that objective measurements were performed by trained technicians in 16 preschools.

In conclusion, our results suggest that airway inflammation assessed by FeNO is significantly correlated to AHR, and indicate that FeNO could serve as a more reliable tool to discriminate preschool children with AHR than other lung function parameters.

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Declaration of interest

The authors report that they have no conflicts of interest. The authors alone are responsible for the content and writing of the paper. This study was supported by a grant from the Korea Healthcare Technology R&D Project, Ministry for Health, Welfare, Republic of Korea (A092076).

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