

Exhaled nitric oxide as a marker of airway inflammation for an epidemiologic study in schoolchildren

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Background: Exhaled nitric oxide (eNO) levels are increased in airway inflammatory disorders, such as asthma.

Objective: We sought to determine whether eNO could serve as a noninvasive marker of allergic airway inflammation for an epidemiologic study in schoolchildren.

Methods: Two hundred seventy-eight schoolchildren aged 10 to 12 years answered a modified American Thoracic Society questionnaire, and eNO levels and pulmonary function were measured. In 246 subjects serum nonspecific IgE levels and levels of IgE specific to house dust mite, cat, cedar, and mold were also measured. Correlation analysis was used to examine eNO levels, nonspecific IgE levels, antigen-specific IgE levels, and pulmonary function. In addition, we compared these variables between subjects with (recurrent wheezers) and without (nonwheezers) recurrent wheeze. Finally, multiple logistic regression analysis was used to find possible predictors for recurrent wheezers.

Results: eNO showed significant positive correlations with nonspecific IgE ($r = 0.62, P < .001$) and mite-specific IgE ($r = 0.74, P < .001$) and weak positive correlations with specific IgE to cat and cedar. Only eNO showed a weak but significant inverse correlation with pulmonary function ($\%FEV_1, P = .035$; $FEV_1/\text{forced vital capacity}, P = .018$). eNO, nonspecific IgE, and mite-specific IgE levels in recurrent wheezers were greater ($P < .001$), and $\%FEV_1$ was less ($P = .06$) when compared with values seen in nonwheezers. Finally, eNO was determined by means of multiple logistic regression analysis to be the best predictor for recurrent wheezers compared with other variables (odds ratio, 11.2; 95% CI, 1.33-94.0).

Conclusion: eNO can be used in epidemiologic studies as a noninvasive marker of allergic airway inflammation in schoolchildren. (*J Allergy Clin Immunol* 2004;114:512-6.)

Key words: Exhaled nitric oxide, wheeze, IgE, atopy, asthma, spirometry

In epidemiologic studies the fundamental issue of how to detect asthma and allergic airway inflammation remains unsolved. Toelle et al¹ suggested that current asthma was

Abbreviations used

ATS: American Thoracic Society
BHR: Bronchial hyperresponsiveness
eNO: Exhaled nitric oxide
FVC: Forced vital capacity
NO: Nitric oxide

defined as the presence of both recent wheeze (12 months before the study) and bronchial hyperresponsiveness (BHR). However, it is unclear whether subjects with recent wheeze but no BHR have substantial airway inflammation. On the other hand, Gibson et al² showed that recurrent wheeze (>2 episodes per year) was a good indicator of eosinophilic bronchitis for community-based surveys because subjects with recurrent wheeze have significantly higher eosinophil counts in induced sputum. These results suggest that recurrent wheeze might be a useful predictor of allergic airway inflammation for epidemiologic studies. However, it might lack specificity because the episode of recent wheeze could occur in subjects with other disorders, such as *Mycoplasma* species, *Chlamydia* species, or respiratory syncytial virus infections.^{3,4}

Exhaled nitric oxide (eNO) levels are increased in airway inflammatory disorders, such as asthma, in adults and children.⁵⁻⁷ eNO in asthmatic patients is mainly produced by inducible nitric oxide synthase expressed in bronchial epithelial cells and some inflammatory cells and is reported to reflect airway inflammation.^{8,9} The fact that eNO levels are increased in subjects with mild-to-moderate asthma,^{5,10} increased after a late asthmatic reaction to allergens,¹¹ and decreased after subjects receive inhaled corticosteroids^{5,12} suggests that eNO is closely associated with airway inflammation.

The aim of this study was to determine whether eNO could be used in epidemiologic studies as a noninvasive marker of allergic airway inflammation in schoolchildren.

METHODS

Study design (population and questionnaire)

Two hundred seventy-eight subjects (138 male and 140 female subjects) aged 10 to 12 years who lived in Fukushima City, Japan, were enrolled in this study. This study was completed before the start of the pollen season (February 2001) to avoid this potential confounding factor. All the subjects and their parents provided

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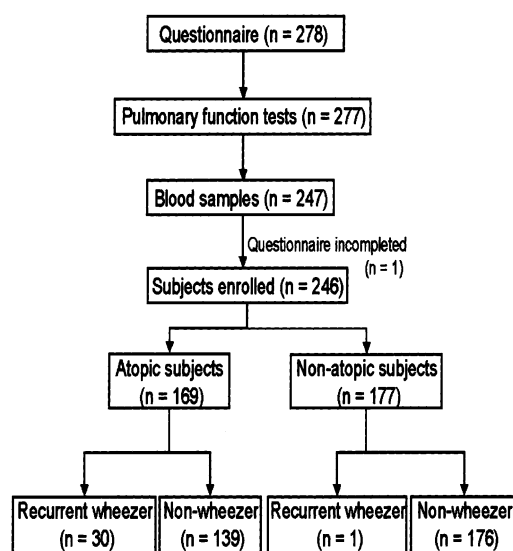


FIG 1. Number of subjects enrolled in this study.

answers to a modified American Thoracic Society (ATS) questionnaire, as published in 1978.¹³ According to this questionnaire, subjects who reported wheeze more than 2 times in the previous 24 months were defined as *recurrent wheezers*, and subjects who had never wheezed were classified as *nonwheezers*. Children with recurrent wheeze were considered to have probable asthma. All subjects provided written informed consent, and the local ethics committee approved this study.

Nitric oxide measurement

eNO was measured according to the ATS recommendations¹⁴ with a chemiluminescence analyzer (model 280i nitric oxide analyzer; Sievers, Boulder, Colo) and defined in parts per billion. The analyzer provides online continuous measurement of nitric oxide (NO) in a single exhalation with a detection range of 0.1 to 500 ppb. Environmental NO was measured before and after each study and never exceeded 5 ppb. All subjects were studied in the sitting position without wearing a nose clip. Subjects exhaled at a constant flow rate (50 mL/s) from total lung capacity to residual volume without breath holding. Subjects maintained a constant mouth pressure (17 cm H₂O) by monitoring a visual display¹⁴ to eliminate contamination from nasal NO. Dead space and nasal NO (which are reflected by the NO concentration peak during exhalation) and NO from the lower respiratory tract (determined by the plateau value after the peak) were recorded automatically by using the manufacturer's software. Three eNO measurements of the plateau phase were obtained, with less than 10% variation. The mean value of 3 successive reproducible recordings was retained for statistical analysis. The eNO measurement was taken before pulmonary function testing.

Pulmonary function tests

Pulmonary function tests were performed with rolling seal spirometers (CHESTAC-11 CYBER S-type; Chest M.I., Inc, Tokyo, Japan). FEV₁ and forced vital capacity (FVC) were measured according to the ATS guideline¹³ by well-trained pulmonary technicians, and the best of 2 or 3 technically acceptable maneuvers reproducible to within 100 mL was retained. Values for FEV₁ and FVC were expressed as percentages of the predicted value for statistical analysis.¹⁵

Allergy screening

Specific IgE to house dust mite, cat, cedar, and mixed molds (*Penicillium*, *Aspergillus*, *Cladosporium*, *Alternaria*, *Candida*, and *Helminthosporium* species) were assessed by using a RAST, and nonspecific IgE levels were determined by means of fluorescence enzyme immunoassay (UniCAP; Pharmacia & Upjohn, Uppsala, Sweden). Antigen-specific IgE concentrations of more than 0.69 UA/mL were regarded as positive RAST results. Subjects were characterized as atopic if they had at least one positive RAST result or a nonspecific IgE level of 250 IU/mL or greater.

Statistical analysis

Statistical analyses were performed with SPSS for Windows (version 8.0; SPSS, Chicago, Ill). eNO, nonspecific IgE, and specific IgE were log normally distributed in the population, and therefore these data were log transformed for statistical analysis. Mean data were expressed as geometric means and 95% CIs. Correlations among eNO, nonspecific IgE, specific IgE, and pulmonary function (%FEV₁, %FVC, and FEV₁/FVC) were made by using Spearman rank analysis. The comparison of each parameter between recurrent wheezers and nonwheezers was made with the Student *t* test. Finally, multiple logistic regression analysis was performed to assess the most useful parameters for differentiating recurrent wheezers and nonwheezers. The odds ratio was used to measure the relative risk for recurrent wheezers, and a 2-tailed *P* value of less than .05 was considered significant.

RESULTS

Characteristics of the subjects

Fig 1 shows the number of subjects enrolled in this study. All the subjects answered the modified ATS questionnaire. Of these, 277 subjects underwent eNO measurement and pulmonary function tests. Furthermore, 247 of 277 subjects provided blood for analysis. Finally, the data from 246 subjects were analyzed for this study because one subject failed to answer the questionnaire completely. Of these 246 subjects, 31 (12.6%) had recurrent wheeze. Moreover, 23 (74.2%) of these 31 subjects were previously given diagnoses of asthma. There were 6 subjects who were treated with a short-acting β_2 -agonist, xanthine, or an antiallergic drug, but they did not use them within 18 hours before the measurements. In addition, none of the subjects had ever received inhaled or oral corticosteroids. On the other hand, 169 (68.7%) subjects had atopy; 30 of them were recurrent wheezers, and 139 of them fell into the nonwheezer group.

Relationship among eNO, serum IgE, and pulmonary function

Fig 2 shows the relationships between eNO and IgE. eNO showed strong positive correlations with nonspecific IgE ($r = 0.623$, $P < .001$) and specific IgE to house dust mite ($r = 0.774$, $P < .001$). In addition, it also showed weak positive correlations to cedar-specific IgE and cat-specific IgE ($r = 0.169$, $P = .008$; $r = 0.297$, $P < .001$, respectively). The average level of eNO was 37.2 ppb (95% CI, 33.1-41.9) in atopic subjects and 15.3 ppb (95% CI, 13.6-17.1) in nonatopic subjects. eNO levels of atopic subjects were significantly higher than those of nonatopic subjects ($P < .001$).

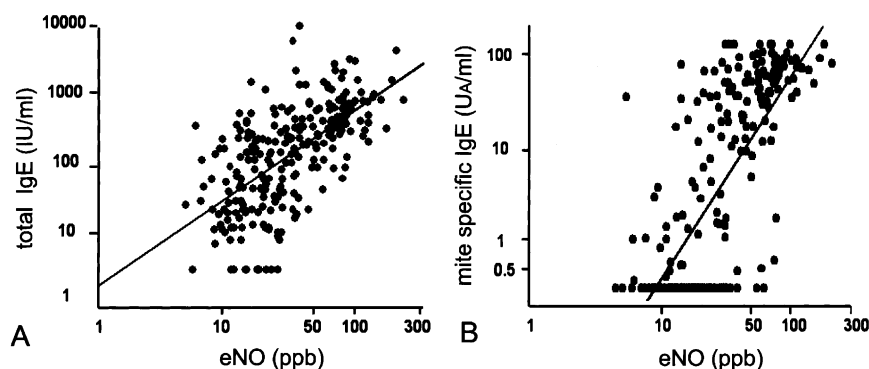


FIG 2. A, Relationships between eNO and total IgE levels (A) and eNO and mite-specific IgE levels (B).

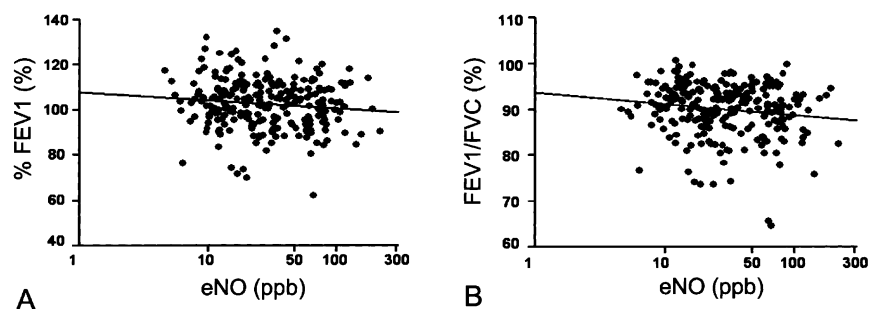


FIG 3. A, Relationships between eNO and %FEV₁ (A) and eNO and FEV₁/FVC (B).

Fig 3 shows the relationships between eNO and pulmonary function. eNO showed weak but significant negative correlations with %FEV₁ ($r = -0.127$, $P = .035$) and FEV₁/FVC ($r = -0.150$, $P = .018$). However, there was no significant correlation between eNO and %FVC ($r = -0.055$, $P = .361$). We could not detect any significant correlation between IgE and pulmonary function (data not shown).

Comparison between recurrent wheezers and nonwheezers

eNO levels, nonspecific IgE levels, and levels of IgE specific to house dust mite were significantly greater in recurrent wheezers ($P < .001$). In addition, %FEV₁ tended to be less in recurrent wheezers ($P = .06$, Table I). However, specific IgE to cedar and cat, which showed significant correlations with eNO, showed no significant difference between recurrent wheezers and nonwheezers.

The average level of eNO was 60.3 ppb (95% CI, 46.8–79.4) in recurrent wheezers, 33.3 ppb (95% CI, 29.4–37.8) in atopic nonwheezers, and 15.1 ppb (95% CI, 13.5–17.0) in nonatopic nonwheezers. All recurrent wheezers except for one subject had atopy, and the eNO level of the subject without atopy in this group was 31.4 ppb. eNO levels of recurrent wheezers were significantly higher, regardless of atopy, than those of nonwheezers ($P < .001$, Fig 4).

Results of multiple logistic regression analysis

Table II shows the final results of multiple logistic regression analysis and the best indicator for differentiating between recurrent wheezers and nonwheezers. Eleven parameters shown in Table II were included in this analysis. Results of the analysis indicated that eNO was the most useful parameter to differentiate recurrent wheezers and nonwheezers (odds ratio, 11.2; 95% CI, 1.33–94.0; $P = .03$).

DISCUSSION

Study of a large sample of schoolchildren shows that a standard method for eNO measurement^{14,16} can be performed easily and properly and used as a noninvasive marker of allergic airway inflammation. The average eNO level was 28.2 ppb (95% CI, 25.4–31.2) in all subjects, 60.3 ppb (95% CI, 46.8–79.4) in recurrent wheezers, and 15.1 ppb (95% CI, 13.5–17.0) in nonatopic nonwheezers. Previously reported eNO levels were highly variable and probably caused by differences in the method used to measure eNO.¹⁷ In this study we measured eNO at a constant flow rate of 50 mL/s, maintaining a constant mouth pressure of 17 cm H₂O according to ATS and ERS recommendations.^{14,16} With this standardized method, we

TABLE I. Comparison of individual parameters

	Recurrent wheezer (n = 31)	Nonwheezer (n = 215)	P value
Age (y)	11.4	11.4	.56
Sex (male/female)	15/16	109/106	.81
Height (cm)	149.1 (146.6-151.6)	149.1 (148.1-150.1)	.98
Weight (kg)	40.9 (38.3-43.5)	42.3 (40.8-43.7)	.34
eNO (ppb)	60.3 (46.8-79.4)	25.2 (22.7-27.9)	<.001
Total IgE (IU/mL)	459 (292-721)	106 (85.1-133)	<.001
Mite-specific IgE (UA/mL)	32.5 (18.6-56.8)	2.39 (1.75-3.26)	<.001
Cat-specific IgE (UA/mL)	0.54 (0.39-0.74)	0.42 (0.37-0.46)	.11
Cedar-specific IgE (UA/mL)	1.54 (0.92-2.57)	0.94 (0.76-1.16)	.10
Mold-specific IgE (UA/mL)	0.36 (0.29-0.45)	0.34 (0.32-0.36)	.50
FVC (% predicted)	99.1 (95.3-103)	98.1 (96.8-99.5)	.60
FEV ₁ (% predicted)	97.2 (93.6-101)	101 (99.7-103)	.06

Data are expressed as geometric means and 95% CIs.

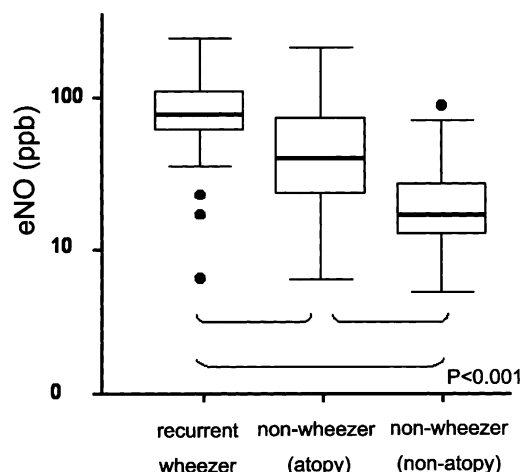


FIG 4. Comparison of eNO levels from recurrent wheezers and nonwheezers with or without atopy. Box plots for eNO levels by subject group are shown. The thick bar is the median value, and the shaded box represents the interquartile range. Outlying points beyond the inner fences are shown individually.

measured eNO levels similar to those seen in previous reports.^{18,19} These results suggest the importance of standardization of the measurement method when eNO is applied to an epidemiologic study.

eNO is strongly correlated with nonspecific IgE and specific IgE to house dust mite. Our results are similar to those of Moody et al,²⁰ who found that increased eNO levels were associated with house dust mite sensitivity in asymptomatic Pacific Islanders. In addition, Henriksen et al²¹ found that there were some correlations between eNO and IgE RAST scores for indoor allergens in subjects who do not have symptoms such as wheeze and BHR, suggesting that atopy itself might increase eNO levels. However, in this study we also observed a weak but significant negative correlation between eNO versus %FEV₁ and FEV₁/FVC and found no correlation between pulmonary function versus total IgE and mite-specific IgE levels. Previous studies revealed some controversy in the relationship between eNO and pulmonary function. Some authors failed to find a relationship between eNO and pulmonary function.^{22,23} However, the sample size was

TABLE II. Multiple logistic regression analysis (association with recurrent wheeze)

	Adjusted OR	95% CI	P value
Log (eNO)	11.2	1.33-94.0	.03
Log (mite-specific IgE)	2.28	0.90-5.78	.08
Sex	1.83	0.75-4.45	.19
Log (mold-specific IgE)	1.21	0.16-9.33	.86
Log (cedar-specific IgE)	1.10	0.54-2.24	.79
Height	1.01	0.93-1.10	.82
Weight	0.99	0.94-1.05	.80
FEV ₁ (% predicted)	0.97	0.93-1.01	.12
Log (total IgE)	0.96	0.26-3.53	.95
Log (cat-specific IgE)	0.92	0.29-2.93	.89
Age	0.56	0.26-1.22	.14

Data are expressed as adjusted odds ratios and 95% CIs.

OR, Odds ratio.

relatively small in these studies, and the standardized method was not applied. On the other hand, a recent study by Strunk et al¹⁰ revealed a relationship between eNO, measured by means of standardized methods, and FEV₁/FVC in subjects with mild and moderate asthma. Furthermore, Djukanovic et al²⁴ reported that some atopic subjects without symptoms and BHR had airway eosinophilic infiltration, degranulation of eosinophils, and subepithelial collagen deposition. From these considerations, it seems reasonable to assume that eNO is linked to atopy and its associated airway pathology, such as allergic airway inflammation.

Recurrent wheeze and BHR have been used frequently to detect asthma and airway inflammation for epidemiologic screening. However, the relationship between eNO and BHR is not understood well. In population studies by Salome et al²⁵ and Henriksen et al,²⁶ levels of eNO were higher in subjects with recent wheeze and BHR. However, once asthma becomes symptomatic, BHR persists in most asthmatic subjects treated with high-dose corticosteroids, even when the degree of airway inflammation improves.²⁷ These facts indicate that although BHR is a good marker of asthma, it might not be a direct marker of airway inflammation.²⁸⁻³⁰ On the other hand, it is unclear whether any significant airway abnormality exists in the subjects with recurrent wheeze. Gibson et al² showed that subjects

with recurrent wheeze have significantly higher eosinophil counts in induced sputum, and Stevenson et al³¹ also demonstrated that atopic children with recurrent wheeze present ongoing airway inflammation represented by eosinophil and mast cell recruitment in bronchial lavage fluid studies. These studies indicate that recurrent wheeze might be a good marker of airway inflammation. In this study we found that eNO levels, nonspecific IgE levels, and levels of specific IgE to house dust mite were greater and %FEV₁ was less in recurrent wheezers. Furthermore, we found that eNO was the most useful marker to detect recurrent wheezers. These results, together with the results of studies mentioned above, suggest that eNO can be used as a sensitive marker reflecting allergic airway inflammation.

Other markers to detect airway inflammation are the number of eosinophils in induced sputum or bronchoalveolar lavage fluid and mucosal eosinophilic infiltration by fiberoptic bronchial biopsies.^{32,33} However, these methods are too invasive and laborious for use in epidemiologic studies and unsuitable for use, especially in children.

In conclusion, the results of this study suggest that eNO can be measured easily and properly in schoolchildren, can be used in epidemiologic studies, and represents a useful noninvasive marker of allergic airway inflammation.

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