



Original Article

Consistently high levels of exhaled nitric oxide in children with asthma

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Abstract *Background:* Exhaled nitric oxide (eNO) levels in children are unstable because they are regulated by many potent factors. The purpose of the current study was to evaluate the reliability of eNO levels between a long interval and other lung functions in normal and asthmatic children.

Methods: Eighty-three elementary school children (aged 11–12 years; male : female, 39 : 44) participated in this study. Lung function, airway resistance and eNO levels were measured twice: the first measurement was in autumn 2007, and the second was one year later.

Results: There were 62 non-asthmatic control children (male : female, 31 : 31) and 21 asthmatic children (male : female, 8 : 13). In both the first and the second examination, the levels of eNO in children with asthma were higher than those in children without asthma. The parameters of lung function and the respiratory resistance in children without asthma showed a good correlation between the results of the first and second examinations. The eNO level in non-asthmatic children showed a good correlation between the two. On the other hand, the peripheral airway parameters of lung function and the respiratory resistance in children with asthma were not correlated between the first and the second examinations. The eNO level in these patients was well correlated between the two examinations.

Conclusions: These data suggest that the eNO level showed good reproducibility in children with and without asthma. The eNO level is therefore considered to be a useful marker for reproducibly evaluating a subject's airway condition.

Key words airway resistance, asthma, children, exhaled nitric oxide, lung function test.

Abbreviations eNO, exhaled nitric oxide; FEV₁, forced expiratory volume in 1 second; FVC, forced vital capacity; IOS, impulse oscillometry system; MMF, maximum midexpiratory flow.

The measurement of exhaled nitric oxide (eNO) has been indicated as a non-invasive marker of airway inflammation, especially in asthma patients.^{1–4} eNO levels are useful for diagnosing childhood asthma and for predicting asthma relapse in children with clinical asthma remission.^{5–7} Recent studies have shown that the eNO level is decreased by anti-inflammatory treatment,⁸ and that the measurement of eNO has been proposed as a method of monitoring the efficacy of treatment in asthmatic patients.³

Recently, useful methods to measure the precise levels of eNO in infants and younger children have been studied.⁹ Previous studies indicate that many factors have a potent influence on eNO levels in children. The effect of acute airway narrowing on eNO levels showed that a methacholine challenge immediately reduces the eNO level (which was not correlated with the per-

centage decrease in forced expiratory volume in 1 s [FEV₁]¹⁰) and that eNO values increase following β_2 -agonist therapy.^{11,12} In addition, the measurement of eNO levels in childhood airway inflammation is affected by many factors, including aging and height.^{13,14}

The present study investigated whether eNO levels in normal and asthmatic children showed good reproducibility. Furthermore, common lung function tests and respiratory resistance in central and peripheral airways were studied in order to evaluate the reliability of eNO measurements in children.

Methods

Subjects

Elementary school children, who were in the sixth grade (ranging from 11–12 years of age) in Maebashi, Gunma prefecture, Japan, participated in this study. The parents of each child completed an American Thoracic Society – Division of Lung Disease respiratory questionnaire in Japanese. If the child had more than three episodes of wheezing and was diagnosed with asthma by a

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physician, the child was determined to have asthma. All asthmatic children participated in the school curriculum and had no attacks when they participated in the study. In addition, at the time of the first visit none of the children were using regularly inhaled steroids, oral steroids, or had a history of smoking, and at the time of the second visit, three asthmatic children used the inhaled steroid fluticasone, and one patient used pranlukast. Ten of 21 asthmatic children (48%) had allergic rhinitis.

The children without asthma included those without asthma or other respiratory diseases. Fourteen of 62 children without asthma (23%) had allergic rhinitis.

Approval was obtained from the hospital ethics committee, and informed parental consent for each child was obtained before the study.

Study design

All of the children's parents were asked for the history of wheezing and respiratory diseases and completed a health check questionnaire. Lung function, airway resistance and eNO level were measured at 15.00–18.00 hours during September to November 2007 in each elementary school.

One year later, all students went to a junior high school. Lung function, airway resistance, and eNO were measured at the same time, with the same methods and by the same staff members during September to November 2008 at their junior high school.

Off-line eNO collection and analysis

The off-line eNO measurement was performed using a modified device (Fig. 1).⁹ The standardized device was modified and a flowmeter with a manometer was attached to monitor the oral pressure and the flow volume. A plastic tube (internal diameter 3 mm and length 23 cm) was connected to a valve and worked and restricted flow. The subjects exhaled through a tube with resistance, keeping a constant pressure of 20–22 cmH₂O leading

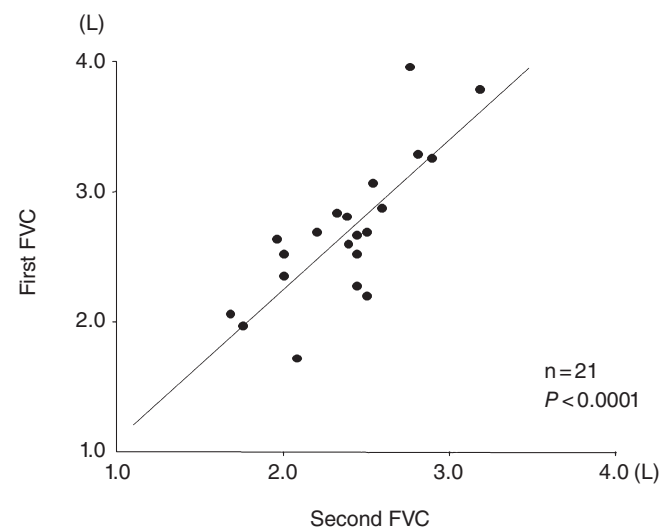


Fig. 1 Correlation between the first and the second forced vital capacity (FVC) results. The central airway parameters of spirometry, FVC, showed a good correlation between the first and the second examination results in children with asthma.

to a constant flow of 50 mL/s. All participants inspired NO-free medical air and performed one single exhalation into an NO inert and impermeable 1.5-L Mylar balloon. The on-line eNO reached a plateau 5 s after starting exhalation in both adults and children,⁹ and the subjects were instructed to continue exhaling for 10 s. After 5 s of exhalation the operator attached the Mylar balloon to the plastic tube and collected the sample for the last 5 s. The sample was measured with an NO analyzer (model 280i metric oxide analyzer; Sievers, Boulder, CO, USA) within 3 h after collection.

Pulmonary function test

The pulmonary function was determined by spirometry using a calibrated computerized pneumotachograph spirometer (MasterScope PC; Jaeger, Hoechberg, Germany). Standard measures of pulmonary function were collected, including the forced expiratory capacity (FVC), FEV₁, maximum midexpiratory flow (MMF), V'₅₀, and V'₂₅/HT. The procedure for all pulmonary function tests included: three normal tidal volume breaths, inhalation to total lung capacity, and the forced maximal exhalation, terminating with a final maximal inhalation. All tests were performed according to the current American Thoracic Society spirometry standards. However, if any baseline measurement was technically unacceptable, then the maneuver was repeated immediately.

Impulse oscillometry system

Respiratory resistance was determined with an impulse oscillometry system (MS-IOS; Jaeger) using the techniques recommended by the manufacturer. Real-time recordings of mouth pressure and flow signals pulsed through a 5- to 20-Hz spectrum, were superimposed over the tracings of the tidal breathing and were displayed on a computer screen. Measurements of resistance at 5 and 20 Hz (R₅, R₂₀, and R₅-R₂₀) and respiratory impedance at 5 Hz (Z₅) were recorded. It was agreed that the value of R₂₀ mainly demonstrates the central respiratory resistance, and the value of R₅-R₂₀ demonstrates peripheral airway resistance.^{11,15}

Data analysis

Statistical analyses and data presentation were performed using the SPSS software package (version 11.0; SPSS, Chicago, IL, USA). The comparisons between the first and the second results and between the non-asthmatic and the asthmatic subjects in spirometry parameters, impulse oscillometry system (IOS) parameters, and eNO levels were made using the Mann-Whitney *U*-test. Correlations between the first and the second data in spirometry parameters, IOS parameters, and eNO level were made using Pearson's test. Data are expressed as the means $\pm$ SD. *P*-values of less than 0.05 were considered to be statistically significant.

Results

There were no significant differences in either height or weight between the children with asthma and those without asthma (Table 1). The values of V'₂₅/HT, and R₅-R₂₀ in asthmatic children were significantly higher than in children with non-asthma

Table 1 Results of lung function test

	No. of children	Height (m)	Weight (kg)	FVC (L)	FEV ₁ (L/s)	MMF (L/s)	V ₅₀ (L/s)	V ₂₅ /HT (L/s/m)
Total								
First	83	1.47 ± 0.1	41.9 ± 10.5	2.50 ± 0.4	2.19 ± 0.4	2.58 ± 0.6	2.91 ± 0.7	0.96 ± 0.3
Second	83	1.53 ± 0.1 [†]	46.0 ± 10.8 [†]	2.87 ± 0.6 [†]	2.57 ± 0.5 [†]	3.04 ± 0.8 [†]	3.38 ± 0.9 [†]	1.14 ± 0.3 [†]
Children without asthma								
First	62	1.48 ± 0.1	42.5 ± 11.4	2.55 ± 0.5	2.23 ± 0.4	2.64 ± 0.6	2.96 ± 0.7	1.00 ± 0.3
Second	62	1.53 ± 0.1 [†]	46.1 ± 11.4 [†]	2.93 ± 0.6 [†]	2.61 ± 0.5 [†]	3.08 ± 0.7 [†]	3.42 ± 0.8 [†]	1.17 ± 0.3 [†]
Children with asthma								
First	21	1.45 ± 0.0	40.2 ± 7.2	2.37 ± 0.4	2.07 ± 0.3	2.38 ± 0.5	2.76 ± 0.5	0.85 ± 0.2 [‡]
Second	21	1.50 ± 0.1 [†]	45.5 ± 8.7 [†]	2.71 ± 0.6 [†]	2.45 ± 0.5 [†]	2.93 ± 0.9	3.27 ± 1.0	1.04 ± 0.3

All data are expressed as the mean ± SD. [†]*P* < 0.01 compared with the first data of each group. [‡]*P* < 0.05 compared with the first data of the children without asthma. FEV₁, forced expiratory volume in 1 second; FVC, forced vital capacity; MMF, maximum midexpiratory flow.

(Tables 1 and 2). The eNO levels in asthmatic children were also significantly higher than those in children with non-asthma.

Children without asthma showed a good correlation between the first and the second results in FVC, FEV₁, MMF, V₅₀, V₂₅/HT, R₂₀, R₅-R₂₀ and Z₅ (Table 3). The eNO levels in children without asthma were correlated between the first and the second examinations.

The spirometry parameters of central airway, FVC and FEV₁ showed a good correlation between the first and the second examinations in children with asthma (Table 3, Fig. 1). The eNO levels in children with asthma also correlated between the first and the second examinations (Fig. 2). On the other hand, the peripheral airway parameters, MMF, V₅₀, and V₂₅/HT and the parameters of respiratory resistance did not show any correlation between the first and the second examinations (Fig. 3).

Discussion

eNO is a good parameter for evaluating airway inflammation. Airway inflammation in asthmatics induces an increase in the eNO level.⁵⁻⁸ On the other hand, there are many factors that have a remarkable effect on the childhood eNO level, including aging, height, allergic rhinitis and anti-inflammatory therapy.^{11,16-18} One can speculate that the eNO level is more unstable in asthmatic patients in comparison to other lung function parameters such as FEV₁, MMF, V₅₀ and V₂₅/HT, because both the degree of airway inflammation itself and the degree of airway narrowing have a potent effect on the individual eNO levels.

However, each asthmatic patient may have a basement level of eNO that is induced by chronic airway inflammation. Therefore, this report evaluated the reproducibility of the eNO level in children with asthma and without asthma, who as a group were strictly limited by age and height, over a one-year interval. The limitation of age and height can minimize these effects.¹⁹ In addition, the limitation of age and area can minimize the opportunity of infection and environmental stimulation.

The current results showed that the eNO levels in asthmatic children were significantly higher than those in non-asthmatic children. This result suggests a strong relationship between airway inflammation and eNO level in children. Many children diagnosed with asthma in elementary school and junior high school experience continuous airway inflammation, even if they can enjoy their everyday school life without an asthma attack.

In children without asthma, the spirometry parameters and the IOS parameters were well correlated between the first and the second examinations. However, the peripheral airway parameters including MMF, V₅₀, V₂₅/HT, and R₅-R₂₀ are thought to be unstable in comparison to the central respiratory parameters in children. The present series demonstrated the reliability of the spirometry, IOS and off-line eNO measurements.⁹ In addition, the fact that these parameters show a good correlation between the first and the second results suggests that each child has a baseline level for both the lung function and eNO level.

Although various methods show different eNO levels, previous reports suggested a cut-off point of 10–50 ppb.^{20,21} In our

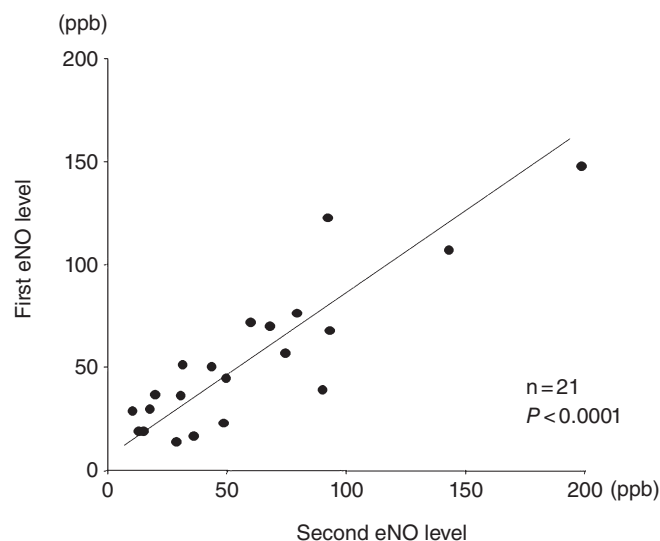
Table 2 Results of impulse oscillometry system parameters and eNO level

	No. of children	R ₂₀ (kPa/L/s)	R ₅ -R ₂₀ (kPa/L/s)	Z ₅ (kPa/L/s)	eNO (ppb)
Total					
First	83	0.41 ± 0.1	0.08 ± 0.1	0.52 ± 0.1	38.7 ± 34.7
Second	83	0.37 ± 0.1 [†]	0.06 ± 0.1 [†]	0.45 ± 0.1 [†]	36.4 ± 27.0 [†]
Children without asthma					
First	62	0.40 ± 0.1	0.08 ± 0.1	0.51 ± 0.1	32.2 ± 25.6
Second	62	0.36 ± 0.1 [†]	0.06 ± 0.1 [†]	0.42 ± 0.1 [†]	30.4 ± 20.4 [†]
Children with asthma					
First	21	0.43 ± 0.1	0.10 ± 0.1 [‡]	0.55 ± 0.2	57.9 ± 49.1 [‡]
Second	21	0.39 ± 0.1	0.10 ± 0.1 [§]	0.52 ± 0.1 [§]	54.0 ± 35.9 ^{‡,§}

All data are expressed as the mean ± SD. [†]*P* < 0.01 compared with the first data of each group. [‡]*P* < 0.05 compared with the first data of the children without asthma. [§]*P* < 0.01 compared with the second data of the children without asthma. eNO, exhaled nitric oxide.

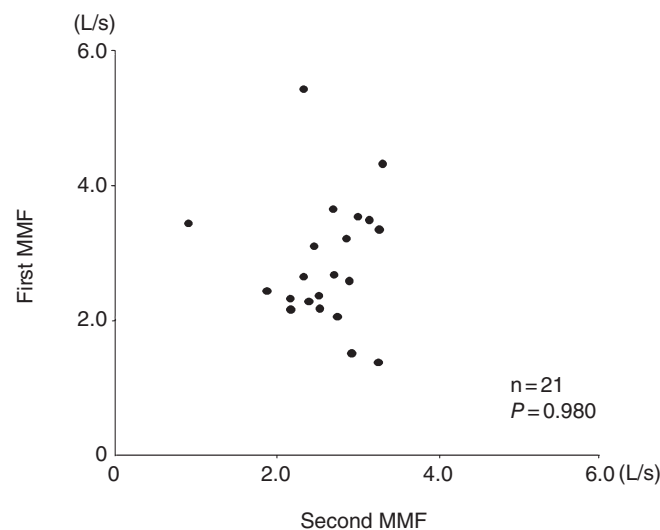
Table 3 Correlation coefficients (CC) and *P*-values of the first and the second results

	Height (m)	Weight (kg)	FVC (L)	FEV ₁ (L/s)	MMF (L/s)	V ₅₀ (L/s)	V ₂₅ /HT (L/s/m)	R ₂₀ (kPa/L/s)	R ₅₋₂₀ (kPa/L/s)	Z ₅ (kPa/L/s)	eNo (ppb)
Total											
CC	0.894	0.968	0.835	0.727	0.416	0.445	0.314	0.449	0.460	0.577	0.781
<i>P</i>	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Child without asthma											
CC	0.883	0.972	0.843	0.777	0.547	0.585	0.390	0.545	0.471	0.688	0.620
<i>P</i>	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Children with asthma											
CC	0.942	0.972	0.794	0.566	0.006	0.032	-0.157	0.216	0.424	0.234	0.872
<i>P</i>	0.000	0.000	0.000	0.007	0.980	0.890	0.495	0.347	0.055	0.307	0.000

eNo, exhaled nitric oxide; FEV₁, forced expiratory volume in 1 second; FVC, forced vital capacity; MMF, maximum midexpiratory flow.**Fig. 2** Correlation between the first and the second exhaled nitric oxide (eNO) level. The eNO level in children with asthma also showed a good correlation between the first and the second examination results.

institution, the cut-off point of the off-line eNO level in normal children is 35 ppb (data not shown). The first trial showed that the eNO levels in 20 of 62 children without asthma were over 35 ppb, and eight of 62 children were over 60 ppb. The current study found that there may be some children who are not diagnosed with asthma, but nevertheless have airway inflammation.

In children with asthma, it is surprising that the eNO level showed a correlation between the first and the second examination results ($P < 0.0001$), whereas the peripheral airway parameters such as MMF, V'_{50} , V'_{25}/HT and IOS showed no correlation between the first and the second examination results. These data suggest that airway inflammation in asthmatic

**Fig. 3** Relationship between the first and the second maximum midexpiratory flow (MMF). The peripheral airway parameters, MMF, did not show any correlation between the first and the second examination results in children with asthma.

children was stable, and that eNO may therefore be a direct indicator, and provide a more accurate result than the lung functional parameters of the peripheral airways.

The eNO levels in childhood asthma significantly correlated with the parameters of peripheral airways. Airway NO originates predominantly from the respiratory mucosa in the bronchioles²² and is mainly produced in peripheral airways, and the lung function parameters of peripheral airways are good indicators of persistent airway inflammation.¹¹ In addition, our previous data suggested that eNO significantly decreases after a methacholine inhalation challenge, and this decrease was primarily dependent on bronchoconstriction in small airways.¹¹ On the other hand, the present study showed that both the central airway resistance (R_{20}) and peripheral airway resistance (R_5 - R_{20}) significantly changed in children with asthma. Consequently, our data suggest that the eNO level is stable at a high level, even in asymptomatic asthmatic children, and the chronic silent peripheral airway inflammation may induce unstable peripheral airway bronchoconstriction.

In this study, 23% of children without asthma and 48% of children with asthma had allergic rhinitis. At the time of the second visit, four subjects had recently used inhaled steroid and leukotriene modifier. Allergic rhinitis and anti-inflammatory drugs have been reported to have an effect on airway NO concentration.^{18,23} However, we included these data because the interval of this study may be too short to evaluate any additional effect of allergic rhinitis, and the number of subjects using these drugs was too small.

In conclusion, chronic airway inflammation induces continuous eNO increases in school children with asthma. This is the first study demonstrating the reproducibility of eNO measurements in children, despite their age and height. The eNO level is a good indicator of airway inflammation, and should therefore be routinely checked to achieve a good control of childhood asthma. In addition, when the eNO level is continuously high, the administration of anti-inflammatory drugs should therefore be considered for such patients.

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