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Airway reversibility and inflammation in stable pre- to late-adolescent asthmatics without long-term control medications

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ABSTRACT

Objective: Pulmonary function and airway inflammation were investigated in stable pre- to late-adolescent asthmatics without long-term control medications and compared with those in currently medicated asthmatics.

Methods: Subjects comprised 34 well-controlled asthmatic children (aged 8.1–18.0 years; group without medication). Flow volume curves before and after inhaling a β_2 agonist, a bronchodilator (BD), were compared and fractional exhaled nitric oxide (FENO) concentrations were measured. All patients were attack-free for at least 12 months prior to testing without the use of asthma medications for at least three months. Fifty-one age-matched stable asthmatics with medications at the time of the present study (group with current medication) underwent the same examinations.

Results: The rate of children whose respiratory function after BD improved by 20% or more in both the central and peripheral airways (High responder at total airways subtype: HTA) was significantly higher in the group without medication than in that with current medication (17.6 and 2.0%, respectively; $p < 0.01$). Furthermore, FEV_{1.0}% pred after BD was significantly lower for HTA than for the low responder subtype in the same group (94.8 ± 3.5 and $104.1 \pm 1.5\%$ respectively, $p < 0.05$). FENO concentrations in the group without medication were high, but not significantly different from those in the group with current medication.

Conclusions: Stable asthmatic children without medication include a certain percentage of those with irreversible airflow limitation possibly due to airway remodeling. The control of daily asthma symptoms with long-term control medications may effectively prevent airway remodeling.

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Flow volume curves; bronchodilator; fractional exhaled nitric oxide (FENO); central airways; peripheral airways; airway remodeling

Introduction

The remission rate of pediatric asthma is higher than that of adult asthma. However, remission is generally defined as the absence of asthma symptoms over a long time period without treatment (1,2), and several subtypes are expected among those in remission. Children with airway inflammation or persistent airflow limitation may be regarded as asthmatic children who are defined as being in remission; however, ideally, neither of these groups may be included among children who are stable after the discontinuation of treatment.

Pulmonary function tests, including spirometry and flow volume curves, are useful parameters of airflow limitation; however, the rate of spirometry being performed is low, even among asthma specialists (3).

Lødrup Carlsen et al. (4) reported that the pulmonary function trajectories of asthmatic pubescent children in remission differed from those of non-asthmatic children, and that it is important to conduct pulmonary function tests for a more precise evaluation of remission states. Wang et al. (5) recently reported that the degree of FEV_{1.0}/forced vital capacity (FVC) ratio impairments was the most accurate predictor of asthma remission, suggesting that the measurement of pulmonary function may be useful for assessing the prognosis of asthma. Among pulmonary function tests, the airway reversibility test (ART) is used to confirm the presence of airflow limitation and evaluate the degree of its irreversibility (6) by comparing pulmonary function before and after the inhalation of bronchodilators (BD), and it is also used to diagnose

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asthma or evaluate the state of asthma control. According to previous findings, a positive ART evaluation is derived when the $FEV_{1.0}$ value improves by 12% or more after the inhalation of BD (7). Furthermore, ART is used to separately evaluate changes in airflow limitation at different airway sites, namely, the central and peripheral airways. We classified patterns of flow volume curve changes before and after BD inhalation into 4 subtypes by employing peak expiratory flow (PEF) and maximum expiratory flow at 50% of the forced vital capacity ($\dot{V}_{50}$) as parameters of the central and peripheral airways, respectively (8). By using this method, we demonstrated that reversible airflow limitation, which is improved by BD inhalation, may be detected, particularly in the peripheral airways, in approximately 50% of pubescent asthmatic children in a stable state (8). However, there is currently no information on airway reversibility in stable asthmatic children not using control medications, among whom children in remission or pre-remission may be included.

Fractional exhaled nitric oxide (FENO) concentrations are an indicator of eosinophilic airway inflammation, and are useful for the diagnosis and management of asthma (9). A normal FENO concentration is less than 20 ppb in children (9). A previous study reported that the initiation of ICS needs to be considered when FENO is 25–35 ppb and also that the use of ICS is recommended when FENO is higher than 35 ppb (10).

In the present study, we focused on stable asthmatic children who were followed up regularly on an outpatient basis after the discontinuation of long-term control medications. These subjects were expected to include a certain percentage of those in clinical remission. We evaluated airflow limitation and its reversibility in these children because it is important to guide these children adequately in their treatment toward remission. FENO concentrations were also measured to investigate its relationship with airflow limitation and a previous history of asthma.

Methods

Subjects

The present study was conducted between July 2011 and July 2017. Thirty-four asthmatic children (24 boys and 10 girls; mean age 12.9 years, median age 13.0 years [8.1–18.0 years]) participated as subjects. They regularly visited Saitama Medical University Hospital or Tone Chuo Hospital on an outpatient basis ($n = 19$ and $n = 15$, respectively). These children were free of asthma attack symptoms for at least 12 months before the initiation of the present study

based on their reports of the complete absence of wheezing and dyspnea at rest and on exertion, and were not receiving any medication to control asthmatic symptoms, including oral steroids or rescue inhalers. Furthermore, they had discontinued using long-term control medications, such as leukotriene receptor antagonists (LTRA), inhaled corticosteroids (ICS), or long-acting β -agonists (LABA), for at least 3 months prior to the test (group without medication).

Data on previous histories of asthma were collected from patient files. Data included [1] the duration of symptomatic asthma (years); the period between the onset of asthma to the last administration of drugs, and [2] the time after the attenuation of symptoms and discontinuation of long-term control medications (months); the period since drug discontinuation. Furthermore, [3] the degree of severity during previous periods with the worst episode of asthma was classified into intermittent, mildly persistent, moderately persistent, and severely persistent based on the JPGL2012 (11) criteria.

As an age-matched control, non-symptomatic asthmatic children with current medications (group with current medication) also underwent the same tests.

This group consisted of 51 stable asthmatic children (29 boys and 22 girls with a mean age of 12.4 years and median age of 12.3 years [8.0–17.4 years]). They also visited Saitama Medical University Hospital or Tone Chuo Hospital ($n = 22$ and $n = 29$) regularly on an outpatient basis. These patients were taking control medications, such as LTRA, ICS, and LABA, daily to prevent the exacerbation of asthma, and had not used systemic steroids for attacks or visited the emergency room for at least three months before examinations.

ART and FENO measurements

ART and FENO measurements were both performed during the regular outpatient visit for asthma. FENO was measured upon arrival at the outpatient clinic, followed by pulmonary function tests before the inhalation of BD. One milligram of salbutamol, a β_2 agonist, was dissolved in 2 ml saline solution and inhaled, and a second pulmonary function test was performed 15 min later. Patients with current medications were tested after the cessation of drug administration for at least 12 h prior to the test.

Pulmonary function tests and assessment of ART

Forced vital capacity (FVC[L]), the forced expiratory volume in one second ($FEV_{1.0}$ [L]), peak expiratory

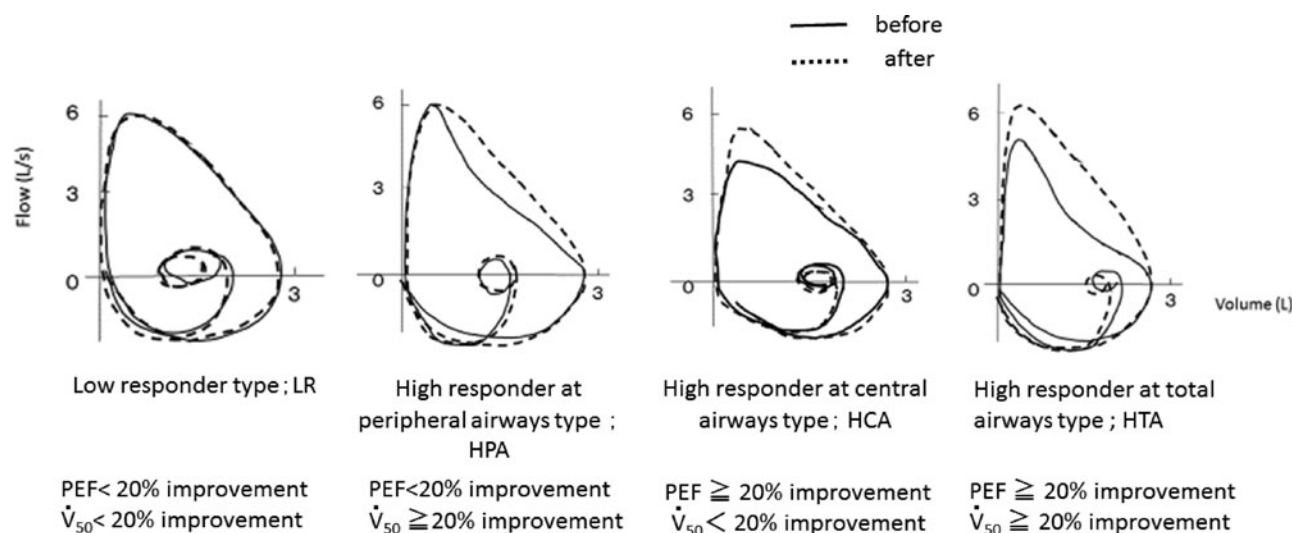


Figure 1. Subtyping of lung function in subjects according to responsiveness to a bronchodilator.

Flow volume curves were compared before (solid line) and after the inhalation of bronchodilators (dotted line). Four different types were observed according to the degrees of changes in PEF and $\dot{V}_{50}$.

flow (PEF[L/S]), and maximum expiratory flow at 50% of the forced vital capacity ($\dot{V}_{50}$ [L/S]) were used as parameters of pulmonary function. The following two points were investigated as changes before and after the inhalation of BD.

The improvement rate of $FEV_{1,0}$

The improvement rate of $FEV_{1,0}$ before and after the inhalation of BD was calculated using the following equation according to a previous study (bronchodilator reversibility; BDR) (7).

$$[(FEV_{1,0} \text{ after inhaling BD} - FEV_{1,0} \text{ before inhaling BD}) \times 100 / FEV_{1,0} \text{ before inhaling BD}] (\%)$$

Patterns of changes in flow volume curves

Patients exhibiting improvements of 20% or more in the parameters of pulmonary function tests before and after BD inhalation were classified as high responders, and those who improved by less than 20% were classified as low responders. Patterns of parameters changes in flow volume curves before and after BD inhalation, having $\dot{V}_{50}$ and PEF as, were arbitrarily classified into four subtypes as shown in Figure 1 (8).

- i. Low responder subtype (LR)
Improvement rates of $\dot{V}_{50}$ and PEF were both less than 20%.
- ii. High responder at peripheral airways subtype (HPA)
The improvement rate of $\dot{V}_{50}$ was 20% or higher whereas that of PEF was less than 20%.
- iii. High responder at central airways subtype (HCA)
The improvement rate of PEF was 20% or higher whereas that of $\dot{V}_{50}$ was less than 20%.

- iv. High responder at total airways subtype (HTA)

The improvement rates of $\dot{V}_{50}$ and PEF were both 20% or higher.

FENO measurement

NIOX-MINO[®] (Aerocrine, Solna, Sweden) was used to measure FENO concentrations with the single-breath online technique recommended by the American Thoracic Society (9): measurements were made using direct expiration for 8 sec or longer while maintaining an exhalation flow speed of 50 m/s and pressure of 5–20 cmH₂O onto the mouthpiece attached to the FENO analyzer.

Statistical analysis

The Mann-Whitney *U* test or Wilcoxon signed-rank test was used to evaluate the significance of differences. The chi-squared test was used to evaluate the significance of differences between groups with and without medication. Relationships between two items were evaluated by Spearman's rank correlation coefficient. Data are expressed as means $\pm$ standard error and as median (minimum - maximum) values. $p < 0.05$ was considered to be significant.

Ethical considerations

The present study was conducted with the approval of the Institutional Review Board of Saitama Medical University Hospital (Approval no. 17-089-2).

Results

Patient backgrounds

Table 1 shows patient backgrounds. No significant differences were observed in age at the examination, gender, the rate of the comorbidity allergic rhinitis (AR), or age at onset between the group without medication and that with current medications. The duration of the period without long-term control medications in the group without medication ranged between 3 and 102 months (mean: 18.5 months).

Changes in parameters of pulmonary function before and after the inhalation of BD

The results of ART in the groups without medication and with current medication are shown in Table 2. The percentages of predicted values (% pred) for FVC and PEF were both higher than 100%, and no significant differences were observed before and after the inhalation of BD in each group. FEV_{1.0}% pred and $\dot{V}_{50}$ % pred before BD inhalation were low for both groups; however, both improved after BD inhalation and reached approximately 100%. No significant differences were observed in any parameters before and after BD inhalation between the 2 groups.

The degrees of BDR in the groups without and with current medication were 5.5 ± 1.0 and $5.2 \pm 0.6\%$, respectively, with no significant differences being observed between the groups.

Patterns of flow volume curve variations

Patients without medication were classified into four subtypes: 14 patients as LR, 13 as HPA, 1 as HCA, and 6 as HTA. The numbers of patients with current medication were classified as follows: 26 patients as LR, 24 as HPA, and 1 as HTA. No patient was classified as HCA. The proportion of HTA was significantly higher in the group without medication than in that with current medication (17.6 and 2%, respectively; $\chi^2 = 6.64$, $p < 0.01$).

Responsiveness to BD was compared by dividing the central and peripheral airways. The proportion of high responders at the central airways (HCA and HTA) was significantly higher in the group without medication than in that with current medication (20.6 and 2%, respectively; $\chi^2 = 8.30$, $p < 0.01$). On the other hand, high responders at the peripheral airways (HPA and HTA) accounted for almost 50% of patients in both groups (without medication, 55.9%; with current medication, 49.0%), indicating no significant difference between the groups.

Table 1. Patient characteristics.

	Age (mean, range; years)	Sex (male:female)	AR (%)	Age of onset (years)	Medication
Without medication (N = 34)	12.9 $\pm$ 0.5 (8–18)	24:10	28 (82.4%)	3.5 $\pm$ 0.5	—
Current medication (N = 51)	12.4 $\pm$ 0.3 (8–17)	29:22	46 (90.2%)	3.4 $\pm$ 0.4	LTRA 15 ICS 7 LTRA + ICS 21 ICS + LABA 3 LTRA + ICS + LABA 5

In the columns 'Age' and 'Age of onset', data are presented as means $\pm$ SEM in the upper panel and as a median and range (in parenthesis) in the lower panel.

No significant differences were observed in age, gender, the complication of allergic rhinitis (AR), or age at the onset of asthma between subjects without medication (Without medication) and those receiving current medications (Current medication). In subjects receiving current medication, leukotriene receptor antagonists (LTRA) alone, inhaled corticosteroids (ICS) alone, LTRA + ICS, ICS + long-acting β -agonists (LABA), and LTRA + ICS + LABA were prescribed for 15, 7, 21, 3, and 5 patients, respectively.

Table 2. Lung function values before and after bronchodilator (BD) inhalation in subjects without medication (without medication) and those receiving current medications (current medication).

		FVC, % pred	FEV _{1.0} , % pred	PEF, % pred	$\dot{V}_{50}$, % pred	BDR(%)
Without medication (N = 34)	Before	101.5 $\pm$ 2.0	96.7 $\pm$ 1.6	109.0 $\pm$ 5.2	79.0 $\pm$ 3.5	—
		101.0 (80.0–128.7)	97.1 (77.1–115.5)	105.1 (57.2–204.2)	80.6 (41.5–139.3)	
	After	100.6 $\pm$ 2.2	101.8 $\pm$ 1.4 ^{††}	115.0 $\pm$ 5.2 [†]	97.2 $\pm$ 3.7 ^{††}	5.5 $\pm$ 1.0
Current medication (N = 51)	Before	99.5 (76.5–127.3)	102.8 (83.2–114.3)	109.4 (75.1–211.7)	93.4 (68.4–168.6)	3.8 (–5.4–20.8)
		102.8 $\pm$ 1.8	97.8 $\pm$ 0.9	108.4 $\pm$ 3.1	82.9 $\pm$ 2.7	—
	After	102.3 (79.1–126.6)	99.0 (80.4–108.5)	108.8 (49.7–148.3)	80.0 (41.5–128.7)	
		102.1 $\pm$ 1.7	102.7 $\pm$ 0.7 ^{††}	111.6 $\pm$ 3.0 [†]	100.4 $\pm$ 2.9 ^{††}	5.2 $\pm$ 0.6
		103.2 (77.2–125.6)	103.1 (88.8–111.6)	115.9 (59.5–155.3)	96.2 (63.7–146.5)	4.6 (–4.0–21.8)

Data are presented as means $\pm$ SEM in the upper panel and as a median and range (in parenthesis) in the lower panel. No significant differences were observed in any of the % predicted (% pred) values of lung function before and after bronchodilator (BD) inhalation between the 2 groups of subjects, including FVC, FEV_{1.0}, PEF, and $\dot{V}_{50}$. FEV_{1.0} – forced expiratory volume in one second; $\dot{V}_{50}$ – maximum expiratory flow at 50% of the forced vital capacity; FVC – forced vital capacity; PEF – peak expiratory flow; BDR – bronchodilator reversibility; [†] $p < 0.05$ and ^{††} $p < 0.01$ compared to the values before BD inhalation in the corresponding group.

Table 3. Lung function values before and after bronchodilator (BD) inhalation in different subtypes in the group of subjects without medication.

		FVC % pred	FEV _{1,0} % pred	PEF % pred	$\dot{V}_{50}$ % pred	BDR(%)
LR (N = 14)	Before	99.8 ± 2.7	100.9 ± 1.7	116.0 ± 9.2	89.4 ± 3.5	—
		103.5 (80.0–114.1)	101.1 (90.0–110.4)	107.7 (81.0–204.2)	89.8 (65.7–109.3)	
	After	98.5 ± 2.7	104.1 ± 1.5 ^{††}	115.3 ± 10.1	97.9 ± 4.5 ^{††}	3.3 ± 0.5
HPA (N = 13)	Before	100.2 (79.0–113.5)	103.7 (95.6–113.0)	107.7 (75.1–211.7)	94.8 (69.0–124.9)	3.1 (0.3–6.3)
		104.2 ± 3.9	96.8 ± 3.0	116.9 ± 6.8	79.2 ± 6.1*	—
	After	96.1 (89.2–128.7)	92.4 (79.3–115.5)	113.9 (68.0–171.7)	75.8 (54.3–139.3)	5.6 ± 1.7
HTA (N = 6)	Before	101.9 ± 4.7	102.0 ± 2.5 ^{††}	117.5 ± 7.3	100.0 ± 7.1 ^{††}	5.5 (–3.4–16.0)
		98.8 (76.5–127.3)	102.0 (83.2–114.3)	110.0 (76.8–181.3)	98.1 (72.4–168.6)	—
	After	101.8 ± 3.7	86.8 ± 3.3**	85.8 ± 7.3	57.3 ± 7.3**	9.3 ± 3.5
		102.7 (91.1–112.0)	87.1 (77.1–98.1)	88.6 (57.2–103.1)	53.3 (41.5–87.2)	9.2 (–3.2–20.8)
		104.5 ± 3.0	94.8 ± 3.5 ^{†,†}	113.7 ± 8.7 [†]	91.5 ± 10.6 [†]	
		107.5 (95.2–113.4)	93.4 (83.9–107.6)	113.6 (88.2–142.6)	83.3 (68.4–139.3)	

Data are presented as means ± SEM in the upper panel and as a median and range (in parenthesis) in the lower panel. When comparing values before BD inhalation, % predicted values of FEV_{1,0} were significantly lower for HTA than for LR ($p < 0.01$), while that of $\dot{V}_{50}$ was significantly lower for both HPA and HTA than for LR ($p < 0.05$ and $p < 0.01$, respectively). Regarding the values obtained after BD inhalation, no significant differences were observed between the LR and HPA subtypes, whereas the % predicted values of FEV_{1,0} remained significantly lower for HTA than for LR ($p < 0.05$).

LR – Low responder subtype; HPA – High responder at peripheral airways subtype; HTA – High responder at total airways subtype; FVC – forced vital capacity; FEV_{1,0} – forced expiratory volume in one second; PEF – peak expiratory flow; $\dot{V}_{50}$ – maximum expiratory flow at 50% of the forced vital capacity; BD – bronchodilator reversibility.

* $p < 0.05$ and ** $p < 0.01$ compared to the value of subjects of the LR subtype before BD inhalation. [†] $p < 0.05$ and ^{††} $p < 0.01$ compared to the value before BD inhalation at the corresponding subtype. ^{†††} $p < 0.05$ compared to the value of subjects of the LR subtype after BD inhalation.

Table 4. Lung function values before and after bronchodilator (BD) inhalation in different subtypes in the group of subjects with current medication.

		FVC % pred	FEV _{1,0} % pred	PEF % pred	$\dot{V}_{50}$ % pred	BDR(%)
LR (N = 26)	Before	103.3 ± 2.5	99.9 ± 0.9	113.2 ± 11.5	90.9 ± 3.7	—
		102.9 (79.1–125.9)	100.3 (88.3–108.5)	111.3 (75.9–148.3)	84.8 (64.5–128.7)	
	After	102.5 ± 2.4	102.7 ± 1.0 ^{††}	115.1 ± 12.8	98.8 ± 4.2 ^{††}	2.8 ± 0.6
HPA (N = 24)	Before	103.7 (78.6–123.6)	103.4 (88.8–110.5)	115.4 (78.6–155.3)	94.7 (63.8–146.5)	2.6 (–4.0–8.2)
		103.3 ± 2.6	95.8 ± 1.4*	108.5 ± 4.9	75.3 ± 3.2*	—
	After	102.3 (79.6–126.6)	97.2 (80.4–106.2)	110.1 (49.7–145.8)	75.2 (41.5–97.7)	7.5 ± 1.0 ^{†††}
		102.6 ± 2.4	102.7 ± 1.1 ^{††}	112.8 ± 5.1 ^{††}	103.5 ± 3.8 ^{††}	6.8 (0.2–21.8)
		103.1 (77.2–125.6)	103.2 (93.3–111.6)	116.2 (59.5–153.6)	104.8 (63.7–126.6)	

Data are presented as means ± SEM in the upper panel and as a median and range (in parenthesis) in the lower panel. When comparing values before BD inhalation, the % predicted values of FEV_{1,0} and $\dot{V}_{50}$ were significantly lower for HPA than for LR ($p < 0.05$) whereas these differences disappeared in comparisons of values after BD inhalation. LR – Low responder subtype; HPA – High responder at peripheral airways subtype; FVC – forced vital capacity; FEV_{1,0} – forced expiratory volume in one second; PEF – peak expiratory flow; and $\dot{V}_{50}$ – maximum expiratory flow at 50% of the forced vital capacity.

BDR – bronchodilator reversibility; * $p < 0.05$ compared to the value of subjects of the LR subtype before BD inhalation; ^{††} $p < 0.01$ compared to the value before BD inhalation at the corresponding subtype; ^{†††} $p < 0.01$ compared to the value of subjects of the LR subtype after BD inhalation.

The values of % pred before and after BD inhalation were compared for each group by subtypes. The results of the group without medication are shown in Table 3 (Data on HCA were omitted from this table because there was only one patient). Before BD inhalation, FEV_{1,0}% pred was significantly lower for HTA than for LR (86.8 ± 3.3 and 100.9 ± 1.7%, respectively; $p < 0.05$). $\dot{V}_{50}$ % pred was significantly lower for HPA and HTA than for LR (79.2 ± 6.1 and 57.3 ± 7.3% vs. 89.4 ± 3.5%, $p < 0.05$ and $p < 0.01$). After BD inhalation, no significant differences were observed in FVC % pred, PEF % pred, and $\dot{V}_{50}$ % pred between the subtypes whereas FEV_{1,0}% pred was still significantly lower for HTA than for LR (94.8 ± 3.5 and 104.1 ± 1.5%, respectively, $p < 0.05$). The degree of BDR was slightly higher for HTA than for LR, and no significant differences were observed between the three subtypes.

The results obtained for the group with current medication are shown in Table 4 (HCA was omitted from the table because there was only one patient). Before BD inhalation, FEV_{1,0}% pred was significantly lower for HPA than for LR (95.8 ± 1.4 and 99.9 ± 0.9%, respectively; $p < 0.05$), $\dot{V}_{50}$ % pred was also significantly lower than LR (75.3 ± 3.2 and 90.9 ± 3.7%, respectively; $p < 0.05$). After BD inhalation, all significant differences in FVC % pred, FEV_{1,0}% pred, PEF % pred, and $\dot{V}_{50}$ % pred between LR and HPA disappeared. BDR was significantly higher for HPA than for LR (7.5 ± 1.0 and 2.8 ± 0.6%, respectively; $p < 0.01$).

Comparison of FENO concentrations

The results obtained for FENO concentrations are shown in Figure 2. No significant differences were

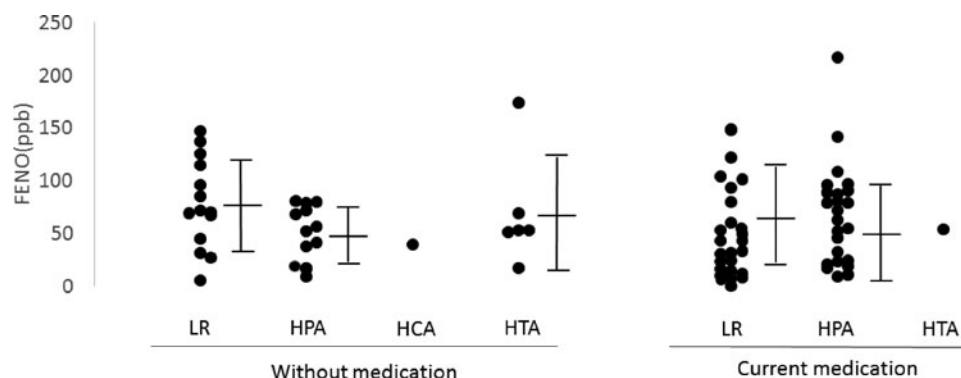


Figure 2. FENO concentrations in subjects without medication and those with current medication.

No significant differences were observed in FENO concentrations between subjects without medication (Without medication) and those receiving current medication (Current medication). No significant differences were observed in FENO concentrations among subjects with different changing patterns in flow volume curves between those without medication and current medication groups. Solid bars indicate the mean $\pm$ standard error.

LR: Low responder subtype, HPA: High responder at peripheral airways subtype, HCA: High responder at central airways subtype, and HTA: High responder at total airways subtype.

observed between the groups without medication and current medication (63.9 ± 6.9 and 58.3 ± 6.4 ppb, respectively). Furthermore, no significant differences were noted in FENO concentrations by subtypes classified according to patterns of flow volume curve variations in both groups with and without current medication.

Comparisons of FENO concentrations based on the intensity of asthma during the most severe period in the past in the group without medication revealed that patients with a persistent severe history had significantly higher FENO concentrations than those with a milder history ($p < 0.05$, Figure 3(A)). An examination of its relationship with disease duration until the discontinuation of medication showed that FENO concentrations increased with longer disease durations (correlation coefficient = 0.40; $p < 0.05$, Figure 3(B)). An examination of its relationship with time since the discontinuation of drug therapy revealed that FENO concentrations significantly increased with longer periods of the discontinuation of treatment until the time of the test (correlation coefficient = 0.42; $p < 0.05$, Figure 3(C)).

Discussion

ART was performed to elucidate airflow limitation and its irreversibility in school-aged children with asthma who are stable without treatment (group without medication), and the results obtained were compared with those from children who are continuously treated with medications (group with current medication). The percentage of predicted values for FVC, $FEV_{1.0}$, PFR, and $\dot{V}_{50}$ after inhaling BD were approximately 100% in both groups. However, the number of children whose

pulmonary function markedly improved following the inhalation of BD at both the central and peripheral airways (the HTA subtype) was significantly higher in the group without medication than in that with current medication. Furthermore, $FEV_{1.0}$ pred after inhaling BD among HTA subjects in the group without medication was 94.8%, which was significantly lower than that in LR subjects in the same group whose % pred was higher than 100%. FENO concentrations in the group without medication were high across the four subtypes classified by ART results, and were similar to values in the group with current medication. In the group without medication, FENO concentrations were significantly higher among subjects with a higher severity of previous symptoms of asthma, and increased with longer disease durations and unmedicated periods.

In both groups with and without current medications, approximately 50% of subjects showed airflow limitation, particularly at the peripheral airways, as indicated by low $\dot{V}_{50}$ in the pulmonary function test before BD inhalation. In addition, in the majority of children whose airflow limitation was confirmed, except for a few, $\dot{V}_{50}$ % pred improved to approximately 100% following BD inhalation, indicating that peripheral airflow limitation is reversible. The present results revealed that, even in children with no symptomatic asthma attacks, airflow limitation may persist and be detected by a pulmonary function evaluation. Therefore, in order to accurately evaluate the pulmonary function of pubescent asthmatic children, it is not sufficient to only assess values before inhalation, changes after inhalation also need to be evaluated. Viložni et al. demonstrated that the responses of spirometric parameters to BD in young children may be

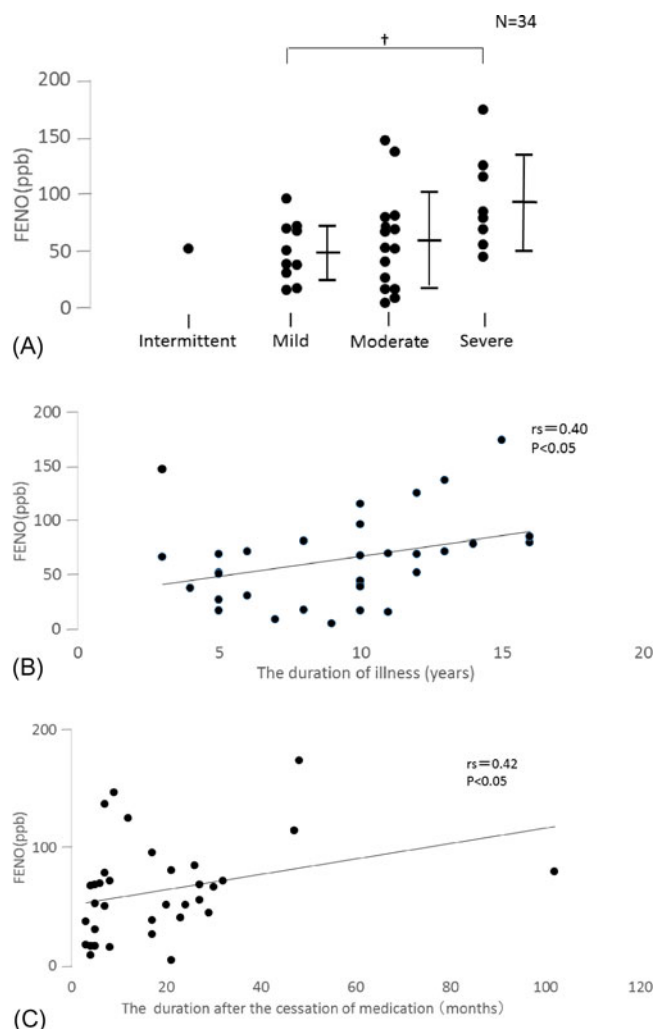


Figure 3. Relationship between FENO concentrations and previous histories of asthma in subjects without medication.

FENO concentrations were significantly higher in subjects with previous histories of persistent severe asthma (Severe) than in those with mild persistent asthma (Mild, $\dagger p < 0.05$). Solid bars indicate the mean $\pm$ standard error. (A). Regarding its relationship with the duration of illness, higher FENO concentrations were observed with increases in the duration of illness ($r_s = 0.4$, $p < 0.05$) (B). Higher FENO concentrations were observed with increases in the duration after the cessation of medication ($r_s = 0.42$, $p < 0.05$) (C).

more sensitive than obstruction detection and may facilitate adjustments to treatment plans (12). Uppala et al. (13) suggested the importance of measuring airway reversibility in asthmatic children currently being treated with low-dose ICS for the proper management of the disease. We demonstrated that ART is a useful method for evaluating the pulmonary function of pubescent asthmatic children without medication. However, it currently remains unclear why reversible airflow limitation, particularly in the peripheral airways, is present in 50% of non-symptomatic children with asthma. Factors affecting airflow limitation in asthma include airway hypersecretion, airway edema, airway smooth muscle contraction, or increased airway resistance by remodeling. Since pulmonary function reversibly improves with the inhalation of β_2

stimulants, the persistent contraction of peripheral airway smooth muscle may be important as a causative factor for airway obstruction in these pediatric patients. However, in addition to less airway smooth muscle being present in the peripheral airways than in the central region, the contraction of peripheral airway smooth muscle does not appear to continue in response to spasmogens in children in a non-episodic state. Another possibility is increased vagal tone in these children, resulting in aggravated airway resistance, which is reversed after the inhalation of a β_2 agonist. Further studies on the mechanisms underlying reversible peripheral airflow limitation in pubescent asthmatic pediatric patients are needed.

In both groups with and without medication, when viewed as a whole, the % pred values of pulmonary

function after the inhalation of BD recovered to normal ranges (approximately 100%) in all parameters. This result suggests that the control of daily asthma symptoms, regardless of the treatment status, with or without medications, is important for maintaining normal pulmonary function and preventing further progression to airway remodeling in pubescent asthmatic children. However, when the results obtained for individual children were examined in detail, the distribution of each subtype in ART differed between the groups with and without medication. In the group without medication, HTA was present in 6 out of 34 subjects (17.6%), but in only 1 (2%) of the 51 patients in the group with current medication; its frequency was significantly higher in the group without medication. Furthermore, comparisons within the group without medication showed that FEV_{1,0}% pred was significantly lower for HTA after BD inhalation than for LR, with the value being approximately 100%. This result indicates that among pediatric patients who are stable and do not require long-term pharmacotherapy for management, a group of subjects without dyspnea have irreversible airflow limitation, namely, airway remodeling. Furthermore, this result was contrary to the prediction that medication may not be necessary and pulmonary function may be ameliorated in children in a stable condition. One reason for this may be that the routine management of pulmonary function was not performed because symptoms were stable in the group without medication. In other words, the routine monitoring of pulmonary function by a peak flow meter or asthma diary in the medicated group is often performed to manage attacks, whereas the need for such routine monitoring is not recognized in the group without medication and, thus, is not generally practiced. Therefore, even if pulmonary function deteriorates, the condition may not be recognized and potentially becomes undertreated. The results of the present study suggest that even patients who are clinically considered to be in remission may require periodic pulmonary function testing, for example, every 6 or 12 months. Furthermore, the present results indicate that irreversible decreases in pulmonary function, namely, remodeling, may have progressed in some pediatric patients in the group without medication. In the present study, in the HTA subtype in the group without medication, FEV_{1,0}% pred did not reach 100%, but remained at a significantly lower value than the LR subtype showing the normal range (as previously described). However, in contrast to the group without medication, only one patient was classified as HTA in the group with

current medication, and all others were classified as LR or HPA. In the group with current medication, FEV_{1,0}% pred after BD was almost 100% in both subtypes, indicating that the continuation of daily prophylactic treatments to prevent the exacerbation of asthma is useful for inhibiting the progression of airway remodeling, and the control of asthma attacks by pharmacotherapy is not merely a symptomatic treatment, it may also improve patient prognoses. Future studies on this issue are required.

In the present study, asthmatic children with no attack episode for at least 12 months and no prophylactic drug use for more than three months before the examination were selected as subjects. When studying children in remission, it would be preferable to limit the subjects to those having a longer no-medication period. However, the reason we had to set the minimum period as a short period was because most of the subjects had the comorbidity of AR. Therefore, many patients were taking oral LTRA as a treatment for AR even if asthma symptoms were not observed for a long period of time, and it was difficult to select patients who maintained a long non-medicated period. A recent study reported that asthma and AR may develop together, and a Japanese study showed that the comorbidity rate of AR in asthmatic children was 58.1% (14). In the present study, the AR comorbidity rate of asthmatic children reached approximately 90% in both groups, and LTRA was prescribed in many patients for the treatment of seasonal AR. However, it is not completely negligible that the symptoms of mild asthmatic attacks, as well as those of AR, were masked during the period of taking this agent as for some subjects in the group without medication.

In ART, in contrast to the other subtypes, LR patients showed only small improvements in $\dot{V}_{50}$ and PEF, indicating a poor response to BD. This result suggests that LR appears to be the most stable type among the 4 subtypes. Contrary, it is also possible that the irreversible fixed airflow limitation had already occurred in this subtype, causing the weak response to BD inhalation. However, this is unlikely because % pred values for pulmonary function parameters (FVC, FEV_{1,0}, and PEF, $\dot{V}_{50}$) before BD inhalation in LR were all 100% or higher, showing a stable state without airflow limitation.

In the present study, FENO concentrations in the group without medication markedly increased to a mean value of more than 50 ppb, suggesting the presence of a high degree of eosinophilic airway inflammation (15). Van den Toorn et al. reported that

FENO concentrations in pediatric patients with the remission of asthma were not significantly different from those in asthmatics currently being treated, and were significantly higher than those in healthy children without asthma episodes (16). The present results support their findings, and revealed no significant difference in FENO concentrations among the 4 different subtypes of flow volume curves. Thus, it currently remains unclear whether the degree of eosinophilic airway inflammation had a direct influence on the extent of airflow limitation or the obstruction site. According to Van den Toorn et al. (17), the inhalation of a salmeterol/fluticasone combination for 3 months improved FENO concentrations, but not FEV_{1.0}% pred in patients in clinical remission. These findings indicate that remodeling had already occurred due to chronic eosinophilic airway inflammation, resulting in no relationship between FENO concentrations and the extent of airflow limitation in the examination.

Among subjects in the group without medication, those with a history of persistent severe asthma in the past had significantly higher FENO concentrations than those with a milder type. FENO concentrations were shown to be higher in patients with severe asthma than in those with mild asthma (15). The present results suggest that persistent eosinophilic airway inflammation exists in children with a history of persistent severe asthma, even without current episodes. FENO concentrations tended to be higher in children with a longer duration of asthma. This indicates that children with severe eosinophilic airway inflammation are more susceptible to prolonged asthma symptoms. FENO concentrations increased as the duration after the discontinuation of asthma control medication became longer. This result indicates that the discontinuation of medications, including ICS, aggravated inflammation in a time-dependent manner in clinically stable children despite the persistence of eosinophilic airway inflammation.

Another reason children without medication had high FENO concentrations may include the existence of AR, a comorbidity in a high number of patients. In the present study, 82.4 and 90.2% of subjects in the groups without medication and with current medication, respectively, had AR. Linhares et al. conducted a systematic review of 18 studies that examined the relationship between AR and FENO (18), and showed that the degree of increases in FENO concentrations in AR patients was approximately the upper limit of healthy subjects or slightly higher. Rolla et al. (19) reported that the mean concentrations of FENO were

68.4 and 24.5 ppb in the group with asthma and AR and that with AR only, respectively. These results suggest that the presence of asthma was the main factor for FENO concentration increases in cases of asthma and AR. Thus, the presence of AR with asthma may have further increased FENO concentrations in the present study, but did not appear to be the primary cause for the marked increase observed.

The present results suggest that various levels of airflow limitation and airway inflammation persist in untreated stable asthmatic children, and that irreversible airflow limitation accompanied by airway remodeling may exist in some pediatric patients. In contrast, asthmatic children well-controlled with medication showed almost normal pulmonary function after BD inhalation, but with various degrees of occlusion in the pulmonary function test before BD inhalation. These results confirmed that periodic monitoring, such as pulmonary function testing, is necessary even for stable children without episodes. They also suggested that the control of asthma by the long-term administration of drugs with anti-inflammatory effects may also help to prevent the progression of irreversible airway remodeling in asthmatic children.

Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this article.

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