Asian Pacific Journal of Allergy and Immunology

ORIGINAL ARTICLE

Determination of fractional exhaled nitric oxide (FENO) reference values in healthy Thai population

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Abstract

Background: Fractional exhaled nitric oxide (FENO) level is directly correlated with airway inflammation in asthma patients. The objective of this study was to define normal FENO levels in healthy Thai volunteers.

Method: This prospective cohort study was conducted in healthy Thai volunteers aged ≥5 years. Demographic and clinical data were recorded and pulmonary function test (PFT) was performed. FENO was measured using a chemiluminescence nitric oxide analyzer.

Results: Seventy-nine healthy Thai volunteers with normal lung function test were included. Mean age of participants was 13 (6-47) years and 58.2% were female. All subjects had no history of allergic respiratory diseases. Mean FENO level increased with age, and the differences between age groups were statistically significant (p=0.001). The highest mean FENO level was 13.6 ppb in the 11-15 year age group, and then the FENO level gradually declined with age. The highest mean FENO level was found in the 18-24.9 body mass index (BMI) group. Significant differences were observed for FENO levels between different height groups (p=0.005) but not between different BMI groups (p=0.46). Fair correlations between FENO levels and body weight, height, FEV1, and FVC were observed. A fair correlation between FENO level and age, FENO level and FEF25%-75% was found only in volunteers ≤15 years of age.

Conclusions: FENO level in healthy Thais increased with age until reaching maximum level (mean FENO 13.6 ppb) in the 11-15 year age group. Significant differences were observed for FENO levels between different age groups and different height groups.

Keywords: fractional exhaled nitric oxide, healthy Thais, pulmonary function test, body mass index

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Introduction

Asthma is a disease that is characterized by chronic airway inflammation, bronchial hyper-responsiveness, and reversible airway obstruction.[1] Effective management of asthma includes understanding of the pathophysiology of the disease, allergen avoidance, asthma education, asthma treatment, and monitoring. Current asthma management objectives include improving treatment of chronic airway inflammation. Asthma control is currently classified into three levels (controlled, partly controlled, and uncontrolled) according to criteria set forth in the Global Initiative for Asthma (GINA) guideline 2011.[1] A fast and easy method to evaluate airway inflammation could be used to assess a patient's level of asthma control and to help monitor therapy with anti-inflammatory drugs

Nitric oxide is a gaseous molecule that is produced by certain cell types (including eosinophils) during an inflammatory response that can be obtained from analysis of gases in exhaled air.[2] After fractional exhaled nitric oxide (FENO) was discovered in 1991,[3] it was found that patients with asthma had higher FENO level[4] and that FENO level was directly correlated with severity of airway inflammation and the number of sputum eosinophils.[5-7] FENO level decreases quickly after administration of anti-inflammatory drugs[8] and the decrease continues during the recovery period.[9,10] Correspondingly, FENO level noticeably increases as airway

inflammation worsens.[11]

There are many factors that may influence FENO level among normal healthy people. Previous studies found that FENO level had no correlation with age, gender, height, pulmonary function test (PFT),[12] body mass index (BMI), or medication adherence.[13] In contrast, subsequent studies refuted those earlier findings and reported association between FENO and height, weight, body surface,[14] and age in children.[15]

Kovesi T, *et al*. studied the relationship between FENO level and race among Canadian children and found that FENO value in healthy Asian-Canadian school children was higher than in healthy white Canadian school children (22.8 *vs*. 12.7 part per billion (ppb), respectively.[16] As such, normal FENO reference values obtained from Caucasian population may not accurately reflect normal FENO reference values in an Asian population. Based on our review of the literature, no previous study has endeavored to determine normal FENO levels in Thai population using a systematic assessment method. Accurate normal FENO reference values for the Thai population would be valuable for interpretation of normal condition, allergic respiratory disease, airway inflammation, treatment monitoring, and for ongoing and subsequent FENO studies in Thailand.

Accordingly, the objective of this study was to define normal FENO levels in healthy Thai volunteers and to identify factors that affect FENO levels.

Methods

Study Design

This prospective cohort study was approved by the Siriraj Institutional Review Board (SIRB) and was registered with the ClinicalTrials.gov registry (NCT02554617; 09/17/15). Written informed consent was obtained from all volunteers and/or their parents. Demographic and anthropometric data were collected, including gender, age, height, and weight. Pulmonary function test results and FENO measurements were recorded and evaluated.

Subjects

This study was conducted in healthy Thai volunteers aged ≥5 years. Healthy volunteers were recruited from the Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok, Thailand. Candidates were excluded if they were pregnant, smoked cigarettes, or had history of asthma, allergic rhinitis, chronic respiratory disease, chronic or systemic disease, or who took regularly prescribed medication. Included volunteers were free from respiratory tract infection within 2 weeks prior to the test, consumed no alcohol within 48 hours before the test, and drank no caffeinated beverages on the day of the test.

Exhaled nitric oxide measurement

FENO was measured by electrochemical technique. An ECO MEDICS CLD 88sp Chemiluminescence NO-analyzer with optional ultrasonic flow meter was used to measure nitric oxide level (ECO MEDICS AG, Dürnten, Switzerland). Nitric oxide was measured in one single exhaled breath. The procedure was conducted in accordance with manufacturer's recommendations and American Thoracic Society/European Respiratory Society (ATS/ETR) recommendations.[17] Tests were performed at the Division of Pulmonary Diseases and Tuberculosis, Department of Medicine, Faculty of Medicine Siriraj Hospital, Mahidol University.

Pulmonary function test

Spirometry was performed by standard method.[18] Baseline FEV1, FVC, and FEF25-75% were shown as absolute value and percent of predicted value.

Statistical analysis

Statistical analysis was performed using SPSS Statistics version 18.0 (SPSS, Inc., Chicago, IL, USA). Characteristics of volunteers, including age, gender, weight, and height, were presented as mean (range) or mean (percentage). Normal FENO level was reported as geometric mean and 95% confidence interval (CI). Comparison between FENO levels and demographic data was performed using one-way ANOVA with Bonferroni method. Factors that were found to affect FENO level were evaluated for strength of association by Pearson correlation analysis. A p-value<0.05 was regarded as being statistically significant.

Results

One hundred healthy volunteers were enrolled. Twenty one of them had abnormal lung function and were excluded from the study. Seventy-nine healthy Thai volunteers with a mean age of 13 (6-47) years were evaluated. Sixty-three percent were ≤15 years of age. Demographic and clinical data are shown in **Table 1**. There were 46 female (58.2%) and 33 male (41.8%) volunteers. The mean BMI was 19.7 kg/m^2.

Table 1. Demographic and clinical characteristics of the studied population (N=79)

Characteristics	Mean (range/%)
Age at time of study (yr)	13 (6-47)
Female	46 (58.2%)
Body weight (kg)	43.4 (17-92)
Height (cm)	145.8 (110-178)
BMI (kg/m^2)	19.7 (13.2-40.28)
Pulmonary function test	
FEV1 (L)	2.2 (0.98-4.26)
FEV1 (%)	97.4 (80-123)
FVC (L)	2.5 (1.02-4.94)
FVC (%)	94.5 (81-121)
FEV1/FVC	0.9 (0.81-1.01)
FEV1(%)/FVC (%)	102.3 (81-115)
PEFR (L)	5.3 (2.09-10.7)
PEFR (%)	100.2 (65-192)
FEF25-75 (L)	2.9 (0.9-5.1)
FEF25-75 (%)	101.8 (27-158)

Abbreviations: BMI, body mass index; FEV, forced expiratory volume in 1 second; FVC, forced vital capacity; PEFR, peak expiratory flow rate; FEF25-75, forced expiratory flow at 25-75%

Geometric means and 95% confidence intervals for FENO reference values according to age are shown in **Figure 1** and **Table 2**. The geometric mean (95% CI) FENO reference value according to age was 9.5 (5.7-18.1) ppb. When volunteers were classified into different age groups (6-10, 11-15, 16-25, and >25 years), the highest FENO level of 13.6 ppb was found in the 11-15 age group, followed by 11.7 ppb in the 16-25 age group. The highest 95% CI of FENO (18.1 ppb) was found in the 11-15 age group. Significant differences were observed between FENO levels in different age groups (p=0.001). Geometric means and 95% CIs for FENO reference values according to height and BMI are shown in **Tables 3 and 4**. Mean FENO levels were significantly higher (p=0.005) in volunteers who were taller than 170 cm, with a maximum FENO level of 15.9 ppb. Significant differences were also observed between FENO levels in different height groups (p=0.005). The highest mean FENO level was 10.8 ppb, 95% CI: 8.6-13.6 in the 18-24.9 kg/m^2 BMI group. No significant difference was found for FENO levels among BMI groups (p=0.46). Geometric mean FENO reference values for male and female subjects were not significantly different [9.7 (7.6-12.6) ppb vs. 9.4 (7.9-11.0) ppb, respectively]. There was no significant association between FENO and gender (odds ratio (OR): 0.7, 95% CI: 0.14-3.4; p=0.6).

Correlation coefficients of factors affecting FENO are shown in **Table 5**. When including all 79 study participants, fair correlations were observed between FENO level and body weight, height, FEV1, and FVC (r=0.31, p=0.005; r=0.40, p=<0.001; r=0.36, p=0.001; and, r=0.36, p=0.001, respectively), but not with age (r=0.11, p=0.32). When including only volunteers ≤15 years of age, fair correlations were observed between FENO level and body weight, height, FEV1, and FVC (r=0.39, p=0.005; r=0.54, p=<0.001; r=0.50, p=<0.001; and, r=0.51, p=<0.001, respectively). FENO levels in the volunteers

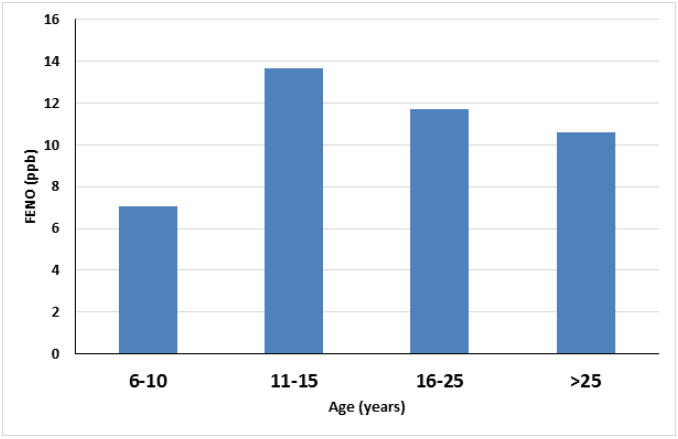

Figure 1. Geometric mean (ppb) of FENO levels in general Thai population

Table 2. FENO reference values for Thais according to age

Age (year)	n	Geometric mean (ppb)	Individual 95% CI	
6-10	33	7.0	5.7-8.7	
11-15	17	13.6	10.3-18.1	
16-25	9	11.7	7.7-17.6	
>25	20	10.6	8.1-13.7	
Total	79	9.5	5.7-18.1	p-value = 0.001

Abbreviations: FENO, fractional exhaled nitrogen oxide; ppb, parts per billion; CI, confidence interval

Table 3. FENO reference values for Thais according to height

Height (cm)	n	Geometric mean (ppb)	Individual 95% CI	
110-120	10	6.3	4.6-8.6	
121-130	11	6.7	4.7-9.5	
131-140	10	7.4	4.9-11.4	
141-150	9	12.3	6.7-22.9	
151-160	23	11.0	8.5-14.1	
161-170	11	11.9	8.7-16.4	
>170	5	15.9	7.9-31.9	
Total	79	9.5	4.6-31.9	p-value = 0.005

Abbreviations: FENO, fractional exhaled nitrogen oxide; ppb, parts per billion; CI, confidence interval

Table 4. FENO reference values for Thais according to BMI

BMI (kg/m^2)	n	Geometric mean (ppb)	Individual 95% CI	
≤18	33	8.8	7.3-10.5	
18-24.9	39	10.8	8.6-13.6	
25-29.9	4	8.1	3.3-20.1	
≥30	3	6.5	2.7-15.8	
Total	79	9.5	2.7-15.8	p-value = 0.46

Abbreviations: FENO, fractional exhaled nitrogen oxide; BMI, body mass index; ppb, parts per billion; CI, confidence interval

Table 5. Correlation coefficients of factors affecting FENO

	Total subjects (n=79)		Subjects aged <15 yrs (n=50)	
	r *	p-value	r *	p-value
Age at time of study (yr)	0.113	0.320	**0.41**	**0.003**
Body weight (kg)	**0.310**	**0.005**	**0.39**	**0.005**
Height (cm)	**0.40**	**<0.001**	**0.54**	**<0.001**
BMI (kg/m^2)	0.070	0.50	0.07	0.59
FEV1 (L)	**0.36**	**0.001**	**0.50**	**<0.001**
FVC (L)	**0.36**	**0.001**	**0.51**	**<0.001**
FEF25%-75% (L)	0.027	0.01	**0.36**	**0.01**

Abbreviations: FENO, fractional exhaled nitrogen oxide; ppb, parts per billion; CI, confidence interval

≤15 years also showed fair correlation with age, and FEF 25%-75% (r=0.41, p=0.003; r=0.36, p=0.01).

Discussion

This study reports for the first time normal FENO reference values in healthy Thai population. The geometric mean FENO reference value increased with age until the age of 15 years. FENO levels correlated with age and height but did not correlate with BMI or gender. Fair correlations between FENO levels and body weight, height, FEV1, and FVC were found.

Mean FENO level of healthy volunteers in our study was lower than the level reported in a previous study.[19] Mean FENO level in our healthy children was comparable to levels found in Canadian (12.7 ppb)[16] and Korean children (12.3 ppb).[20] However, the mean FENO level in our study was much lower than the level found in Asian-Canadian children (22.8 ppb).[16] In our adult volunteers, the mean FENO level (11.1 ppb) was lower than adult rates reported from two studies from the United States (14.1 and 13.3 ppb).[19, 21]

There have been inconsistencies in the data reported about association between FENO and gender, age, body weight, and height. Studies from Europe, the United States, and Canada reported that FENO levels increased significantly with increasing age in school-age children.[15,16] ATS/ERS guidelines state that FENO is related to age and body surface area in children less than 11 years of age.[22] Studies from the United States[19,21] reported that geometric mean FENO level in men was higher than in women. In our study and similar to a study from Korea,[20] gender did not affect FENO levels. When we categorized volunteers by age group, we found that age affected FENO levels. Studies in pediatric populations[16,20] reported no significant relationship between FENO and BMI, which was similar to our study. Our study found association between FENO level and height, which was similar to a study from Canada,[16] and a fair correlation between FENO level and body weight, which was in contrast to previous studies from Europe, the United States, and Canada.[15,16] We also found fair correlations between FENO level and both FEV1 and FVC, which were similar to findings from a study from Canada.[16] Several previous studies demonstrated that FENO measurement is reproducible, noninvasive, and suitable for eosinophil airway inflammation evaluation in adults and children.[4-7] The sensitivity and specificity of FENO level in preschool asthma children were both high (86% and 92%, respectively).[23] However, data regarding FENO levels in normal healthy population are limited.

Our study has a number of strengths. First, this was the first study to evaluate FENO reference values in a healthy Thai volunteer population. Secondly, healthy volunteers in our study were carefully selected to ensure absence of atopic respiratory diseases, absence of other chronic diseases, and absence of medication usage that could adversely affect data and findings. Thirdly, ATS/ERS guidelines for FENO measurement were strictly followed by an experienced technician. Moreover, volunteers were advised to strictly avoid certain foods and beverages before the test that could adversely affect FENO levels.

The present study also has some mentionable limitations. The number of study volunteers in some age groups was quite small. Although the highest 95% CI of FENO reference value by age was 18.1 ppb, we could not conclude that it would be the cut-off level for normal Thai population or that it could be used to diagnose atopic volunteers. A larger study with a sufficient number of volunteers in each age group should be conducted to confirm a FENO cut-off value. Further studies should also be conducted to compare FENO levels between healthy and atopic Thai populations. In our study, some enrolled participants had baseline FEV1 below 80% of predicted values. These participants with abnormal lung function test were excluded from the evaluation since they might possibly have silent bronchial hyper-responsiveness, and this may have affected FENO reference values. Lastly, the maximum age of volunteers was 47 years, so our reference FENO level can only be considered valid in healthy Thais ≤47 years of age. Studies in a full range of age groups should be undertaken so that FENO reference values in normal healthy population are available for all age groups.

Conclusion

FENO level in healthy Thais increased with age until reaching maximum level (mean FENO 13.6 ppb in the 11-15 year age group. Significant differences were observed for FENO levels between different age groups and different height groups.

Acknowledgements

The authors gratefully acknowledge the healthy Thai volunteers that agreed to participate in this study. The authors also would like to thank Ms. Julaporn Pooliam for assistance with statistical analysis.

Conflict of interest declaration

The authors hereby declare no personal or professional conflicts of interest regarding any aspect of this study.

Funding disclosure

This study was supported by a Chalermprakiat Grant from the Faculty of Medicine Siriraj Hospital, Mahidol University and by NSTDA Chair Professor grant (P-1450624) funded by Crown Property Bureau.

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