Study of nasal exhaled nitric oxide levels in diagnosis of allergic rhinitis in subjects with and without asthma

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Background: The measure of fractional exhaled nitric oxide (FENO) in the airways is a useful tool to guide the diagnosis and titration of inhaled corticosteroids in patients with asthma. However, its role in diagnosis of allergic rhinitis (AR), especially in subjects with asthma, is not well established.

Objective: To study the cutoff of nasal FENO in the diagnosis of subjects with AR and AR-asthma compared to age-matched subjects without AR or asthma and its correlations with the clinical and functional characteristics.

Methods: The study was cross sectional and descriptive. Subjects were grouped into control subjects, AR, and AR-asthma, based on the inclusion criteria. Exhaled NO (nasal FENO, bronchial FENO, and alveolar concentration of NO) was measured by multiple flow electro-luminescence device.

Results: Six hundred twenty-eight subjects were included: 217 control subjects (children: n=98, 10±4 years; adults: n=119, 50±16 years), 168 subjects with AR (children: n=54, 10±3 years; adults: n=114, 49±15 years), and 243 subjects with AR-asthma (children: n=115, 10±3 years; adults: n=128, 51±14 years). Nasal peak inspiratory flow and peak expiratory flow were lower in subjects with AR and AR-asthma than in control subjects (P<0.001 and P<0.001, respectively). Nasal FENO levels were significantly higher in subjects with AR and AR-asthma than in control subjects (1614±629 and 1686±614 ppb vs 582±161 ppb; P<0.001 and P<0.001, respectively). In subjects with AR non-asthma, the cutoffs of nasal FENO for those diagnosed with AR were 775 ppb in children, 799 ppb in adults, and 799 in the general population (sensitivity: 92.68%, 92.63%, and 92.65%, respectively; specificity: 91.67%, 95.00%, and 96.87%, respectively). In subjects with AR-asthma, the cutoffs of nasal FENO were higher, especially in asthma children (1458 ppb; sensitivity: 72.97% and specificity: 95.83%).

Conclusion: Nasal FENO measurement is a useful technique for the diagnosis of AR in subjects with and without asthma.

Keywords: nitric oxide, NO, FENO, nasal FENO, allergic rhinitis, asthma

Introduction

Allergic rhinitis (AR) is a very common disease that affects approximately 25% of the world’s population and its prevalence continues to increase.1 In the US, 20–40 million people are diagnosed with AR and some data suggest approximately 40% of children have AR.2 In Europe, the estimated prevalence of AR is 23% of the population.3 In Vietnam, the prevalence of AR in the general population (GP) has not been reported until now. However, unpublished data presented at Vietnamese Ear–Nose–Throat (ENT) Congress in 2016 showed that the prevalence of AR in local Vietnamese population varied from 14.9% to 39.7%. The symptoms of AR include...
rhinorrhea, nasal pruritus, sneezing, and nasal congestion. AR may be seasonal, perennial, or intermittent, depending on exposure and sensitization. AR is often associated with allergic symptoms of the eyes (conjunctivitis), skin (eczema), and lower airways (asthma). Most patients with asthma have concurrent AR and up to 40% of AR patients have asthma. Recent studies showed that the level of asthma control in patients with AR and asthma is correlated with the severity of AR.

The high prevalence of AR in patients with asthma is often referred to as the one airway theory since both are characterized by influx of eosinophils, mast cell degranulation, and upregulation of Th2-related cytokine production from the lymphocytes. For this reason, patients with AR with or without asthma frequently are assessed for airway hyperresponsiveness (challenge with reversibility), spirometry for airway obstruction, skin testing for hypersensitivity to allergens, total and specific IgE, and fractional exhaled nitric oxide (FENO) measurement.

Nitric oxide (NO) is produced mainly by the nasal and bronchial epithelial and inflammatory cells under the control of inducible NO synthase. In subjects with AR, nasal FENO is triggered principally by airborne allergens (pollens, house dust mite, insects, or pets). Similar to the lower airway, the concentration of nasal FENO can be measured by constant aspiration or expiratory flows by nasal mask. Studies demonstrated that in healthy subjects and in subjects with AR, the values of nasal FENO are dependent on aspiration and expiratory flow rate. However, in subjects with AR, especially who have associated asthma, the diagnosed values of nasal FENO are not well defined.

This study was planned to determine the cutoffs of nasal FENO in Vietnamese subjects with AR or AR-asthma, compared to healthy controls, and the correlation between nasal FENO and the clinical and functional characteristics in these patients.

Subjects and methods

Subjects

Subjects more than 5 years old living in Vietnam, who came to the Clinical Research Center of Lam Dong Medical College for diagnosis and treatment of AR or for asthma and met the inclusion and exclusion criteria, were included in this study after they signed an Institutional Review Board approved consent. For subjects under 18 years, the parents or guardians signed the informed consent. This study has been approved by Lam Dong Medical College Institutional Review Board.

Exclusion criteria

Study subjects having one of the following criteria were excluded from the study: acute or chronic cardiorespiratory diseases (acute myocardial infarction, severe coronary disease, chronic heart failure, chronic obstructive pulmonary disease), severe asthma exacerbations needing systemic corticosteroid therapy at screening, AR or asthma treated currently with nasal or inhaled corticosteroids, septal deviation or nasal polyp diagnosed by ENT doctors, and subjects unable to perform functional tests or measurement of FENO.

Inclusion criteria

AR: the clinical diagnosis of AR (made by ENT doctors) was made when patients had one or more of the symptoms (nasal congestion, nose running, nasal itching, or sneezing) lasting more than 4 days per week and occurring when exposed to allergens (dog or cat allergen, pollen, fungus, house dust mites) in the environment or workplace (exposure to certain chemicals, irritants, or allergens at work).

Asthma: the clinical diagnosis of asthma was based on the Global Initiative for Asthma guidelines with a history of respiratory symptoms such as wheeze, shortness of breath, chest tightness, and cough that varies over time and in intensity. The spirometry confirmed the reversibility of airway obstruction.

Control: age-matched healthy subjects were included in this cross-sectional and control study after they or their parents or guardians (for subjects under 18 years old) signed an approved consent. Healthy subjects were excluded from the present study if they had one of the following criteria: other allergic diseases, symptoms of AR or asthma, or positive skin prick test.

Methods

Study design

This study was a cross-sectional and descriptive study. The study subjects were classified as control, AR, and AR-asthma groups according to the inclusion and exclusion criteria. All data on medical history, family history, clinical examination, skin tests, lung function, and exhaled nitric oxide parameters were collected for statistical analyses.

Laboratory techniques

Skin prick test (Stallergenes, London, UK): negative control was 0.9% saline solution and positive control was 1 mg/mL of histamine. Six respiratory allergens including Dermatophagoides pteronyssinus (Dp), Dermatophagoides farinae (Df), Blomia tropicalis (Blo), dog dander, cat...
dander, cockroach, *Phoenix dactylifera*, *Alternaria* spp, and mixed pollens (*Dactylis glomerata*, *Phleum pratense*, *Lolium perenne*) were used for testing. Percutaneous skin test was considered positive when the wheal size exceeded the negative control by 3 mm.

Lung function testing was done using Koko Spirometer (nSpire Health, Inc., Longmont, CO, USA) or with Body Box 500 (Medisoft, Sorinnes, Belgium) for whole-body plethysmography. The reversibility of forced expiratory volume in 1 second (FEV₁) was evaluated 15 min posttreatment with 200 µg (for children) or 400 µg (for adult) salbutamol. The reversal was positive when there was an increase of FEV₁ ≥12% and 200 mL.

Nasal peak inspiratory flow (PIF) and nasal peak expiratory flow (PEF) were measured with Mediflux Device (Mediflux, Paris, France).

### Exhaled NO

Nasal exhaled NO (nasal FENO) was measured with constant aspiratory flow using the Hypair FeNO+ Device (Medisoft), which is an electrochemical based analyzer. The procedure is as follows: 1) patient connects a catheter to one of his/her nostrils; 2) air from the nasal cavity is continuously analyzed by the NO electrochemical sensor; and 3) throughout the analysis, patient breathes through an expiratory brake, so that the velum is closed to prevent any contamination of nasal air with bronchial air. The aspiration flow rate was 100 mL/second through a nasal catheter.

For the airways, bronchial FENO and alveolar concentration of NO (CANO) were measured at multiple flow rates (50, 100, 150, and 350 mL/second). Technical measurement of exhaled NO was taken according to manufacturer’s instructions, as recommended by the American Thoracic Society/European Respiratory Society guideline and the results of exhaled NO had been reported by Hypair FeNO+ Expair Software (Medisoft).15

### Statistical analyses

SPSS 22.0 software (IBM Corporation, Armonk, NY, USA) was used to analyze these data. Categorical variables were expressed as numbers or percentages. Continuous variables were presented as mean ± standard deviation (SD). Normal distribution was tested using skewness–kurtosis. Regression analysis was used to measure the correlation between nasal FENO and continuous variables, with the correlation coefficient *R* of Pearson for normal distribution variables (PIF, PEF, bronchial FENO, and CANO) and of Spearman for non-normal distribution variables (age, height, weight, body mass index [BMI], AR symptoms, and lung function parameters). The pair wise comparison of mean was performed using Mann–Whitney *U* test, and comparison of more than two groups was done with Kruskal–Wallis test. One-way analysis of variance was used to determine whether there were any statistically significant differences between the mean values of three or more independent (unrelated) groups. Youden index with area under the curve (AUC) was used to determine the cutoff of nasal FENO for diagnosis of AR in patients with or without asthma. MedCalc software (Ostend, Belgium) with DeLong method was used to compare the AUC between groups.

### Results

#### Clinical and functional characteristics of the study subjects

From January 2015 to May 2016, 628 subjects were included in the present study, including 217 control subjects, 168 subjects with AR (AR non-asthma), and 243 subjects with AR-asthma. The anthropometric and clinical and functional characteristics of the study subjects are presented in Table 1. There were no significant differences between the three groups in mean age, male to female ratio, or BMI. The AR symptoms were significantly different between subjects with AR vs those with AR-asthma, with predominance of nasal congestion in the AR-asthma cohort (92% vs 75%, *P*<0.05; Table 1). There was no significant difference in the percentage of positive skin tests between subjects with AR and those with AR-asthma (Table 1). The FEV₁ was significantly lower in subjects with AR-asthma than in control subjects and subjects with AR non-asthma (77%±23% vs 97%±11% and 93%±12%, *P*<0.001 and *P*<0.01, respectively; Table 1). Nasal PIF and PEF were significantly lower in subjects with AR and AR-asthma than in control subjects (86±22 and 89±37 L/min vs 134±33 L/min, *P*<0.01 and *P*<0.01; and 134±50 and 113±33 L/min vs 175±48 L/min, *P*<0.05 and *P*<0.01, respectively; Table 1). The levels of nasal FENO were significantly higher in subjects with AR and AR-asthma than in control subjects (1614±629 and 1686±614 ppb vs 582±161 ppb; *P*<0.001 and *P*<0.001, respectively).

#### Correlation between nasal FENO, clinical and functional characteristics, bronchial FENO, and CANO

There were no significant correlations between nasal FENO and anthropometric characteristics (age, height, weight, and BMI; Table 2). However, there were significant correlations between nasal FENO and clinical symptoms in AR and AR-asthma cohorts (Table 2). While the symptoms of sneezing...
and/or nasal itching were strongly correlated with nasal FENO in subjects with AR ($R=0.742, P=0.0029$; Table 2), the symptom of nasal congestion was strongly correlated with nasal FENO in subjects with AR-asthma ($R=0.786, P=0.0034$; Table 2). There were moderate negative correlations between nasal FENO, PIF, and PEF in subjects with AR and AR-asthma ($R=-0.482, P=0.001$ and $R=-0.467, P=0.005$; $R=-0.492, P=0.012$, respectively; Figure 1). However, there were no significant correlations between nasal FENO, bronchial FENO, and CANO (Table 2).
The results of the present study showed that in subjects with AR, the cutoffs of nasal FENO with the highest Youden index were 775 ppb in children, 799 ppb in adults, and 799 ppb for the GP, with high sensitivity (92.68%, 92.63%, and 92.65%, respectively) and specificity (91.67%, 95.00%, and 96.87%, respectively; Table 3). However, in subjects with AR-asthma, the cutoffs of nasal FENO for diagnosis of AR were higher than those in subjects with AR non-asthma, especially in asthmatic children (1458 ppb) with lower sensitivity (72.97%) and high specificity (95.83%; Table 3).

**Comparison of diagnostic values of AUC of nasal FENO**
The AUC values of diagnostic values of nasal FENO were not significantly different between children with AR and with AR-asthma (0.947±0.0317 vs 0.957±0.0256, P=0.8072; Figure 2), adults with AR and with AR-asthma (0.956±0.0195 vs 0.992±0.0086, P=0.0922; Figure 2), and GP (0.953±0.0167 vs 0.956±0.0154, P=0.9024; Table 4).

**Discussion**
The results of our study showed that: 1) the levels of nasal FENO in subjects with AR and AR-asthma were significantly
higher than in control subjects; 2) the levels of nasal FENO were significantly correlated with AR symptoms and PIF and PEF; 3) the cutoffs of nasal FENO for diagnosis of AR-asthma were higher than in subjects without asthma; and 4) the sensitivity and specificity of the nasal FENO cutoffs for AR diagnosis were not significantly different.

In healthy subjects, the concentration of nasal FENO is higher than that in the lower airways as measured by aspiration (350–750 vs 5–25 ppb). Previous studies demonstrated that in the upper airways, exhaled nasal NO was produced mainly from paranasal sinus. However, a recent study demonstrated that the nasal exhaled NO was obtained not

<table>
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<tr>
<th>Table 3</th>
<th>Cutoff of nasal FENO for diagnosis of AR classified by population</th>
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<tr>
<td><strong>Nasal FENO</strong></td>
<td><strong>Cutoff, (ppb)</strong></td>
</tr>
<tr>
<td><strong>AR</strong></td>
<td></td>
</tr>
<tr>
<td>Children</td>
<td>775</td>
</tr>
<tr>
<td>Adults</td>
<td>799</td>
</tr>
<tr>
<td>GP</td>
<td>799</td>
</tr>
<tr>
<td><strong>AR-asthma</strong></td>
<td></td>
</tr>
<tr>
<td>Children</td>
<td>1458</td>
</tr>
<tr>
<td>Adults</td>
<td>990</td>
</tr>
<tr>
<td>GP</td>
<td>1271</td>
</tr>
</tbody>
</table>

**Abbreviations:** AR, allergic rhinitis; CI, confidence interval; FENO, fractional exhaled nitric oxide; GP, general population; LR, likelihood ratios; ppb, parts per billion.

<table>
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<th>Table 4</th>
<th>Comparison of diagnostic values of nasal FENO between subjects with AR and AR-asthma</th>
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<tr>
<td><strong>Comparison</strong></td>
<td><strong>AUC</strong></td>
</tr>
<tr>
<td>Children</td>
<td></td>
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<tr>
<td>AR</td>
<td>0.947</td>
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<tr>
<td>AR-asthma</td>
<td>0.957</td>
</tr>
<tr>
<td>Adults</td>
<td></td>
</tr>
<tr>
<td>AR</td>
<td>0.956</td>
</tr>
<tr>
<td>AR-asthma</td>
<td>0.992</td>
</tr>
<tr>
<td>GP</td>
<td></td>
</tr>
<tr>
<td>AR</td>
<td>0.953</td>
</tr>
<tr>
<td>AR-asthma</td>
<td>0.956</td>
</tr>
</tbody>
</table>

**Note:** AUC comparison with DeLong method.

**Abbreviations:** AR, allergic rhinitis; AUC, area under the curve; CI, confidence interval; FENO, fractional exhaled nitric oxide; GP, general population; SE, standard error.
Nasal exhaled NO in diagnosis of AR

The measure of nasal exhaled NO is a feasible and useful method for the diagnosis of AR in healthy subjects and subjects with asthma. The cutoffs of nasal FENO for the diagnosis of AR are different in subjects with asthma from those of subjects without asthma. Hence, it is necessary to perform more studies in a large number of subjects to clarify the role of nasal exhaled NO in the diagnosis of AR in the GP and in subjects with asthma.
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Disclosure

The authors report no conflicts of interest in this work.

References


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