Study of Nasal Fractional Exhaled Nitric Oxide (FENO) in Children with Allergic Rhinitis

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1. Introduction

In the upper airway, exhaled nitric oxide (NO) is produced mainly from the rhin-sinusitis mucosa. It can be measured by non-invasive techniques using devices with chemiluminescence or electroluminescence methods [1–3]. The main source of nasal NO is consistently generated from the nasal mucosa and perinasal sinus epithelium, where inducible nitric oxide synthase (iNOS) is present. In the upper airway, the role of nasal NO has been described as regulating airway function, providing non-specific protection against...
infection related to its destructive property. Nasal NO also contributes to upper airway protection due to its role in regulating ciliary motility, and low nasal NO levels are usually associated with decreased upper airway ciliary function [4]. Nasal NO has been proposed in the hypothesis of humidifying and warming of inhaled air through the nasal passage.

The alteration in nasal fractional exhaled NO (nasal FENO) levels has been described previously in various diseases such as allergic rhinitis (AR), primary ciliary dyskinesia (PCD), cystic fibrosis, and sinusitis [5–8]. Therefore, the measure of nasal FENO is now considered a useful biomarker in clinical practice for patients with rhino-sinusitis diseases. In patients with PCD, nasal FENO measurement is routinely performed for screening this genetic disorder [9]. In patients with AR, nasal FENO has been used to manage the disease in the same manner as FENO in patients with asthma [10]. The increased iNOS expression and activity due to contact with airborne allergens induces the production of nasal FENO in patients with AR. While the correlation between exhaled NO and lower airway inflammation in asthmatic patients due to eosinophils has been demonstrated, the application of nasal FENO measurement in patients with AR is relatively complex and remains controversial.

Hence, this study was conducted to evaluate (1) the correlation between nasal FENO and anthropometric characteristics, symptoms of AR, and nasal peak flows in children without and with AR; and (2) the cut-off of nasal FENO for diagnosis of AR in symptomatic children.

2. Methods

2.1. Patients

Patients with a diagnosis of AR were included in the current study when they were referred to the Clinical Research Center of Lam Dong Medical College for measuring nasal FENO and a skin-prick test. The present study was approved by the IRB of Lam Dong Medical College, Dalat, Vietnam (ID: CDYTLD.NCKH.03.2018); signed written informed consent was obtained from all the study subjects. The study followed the principles of the 1964 Declaration of Helsinki.

2.1.1. Inclusion Criteria

Patients <18 years old with AR symptoms (nasal congestion, runny nose, nasal itching, or sneezing) lasting more than 4 days per week and for more than 4 consecutive weeks were classified into the AR group.

2.1.2. Exclusion Criteria

The exclusion criteria were one of the following features: severe cardiorespiratory disease, AR treated with oral or local corticosteroids, septal deviation or nasal polyp diagnosed, and upper or lower airway infection in the past 15 days; subjects unable to undergo the functional laboratory testing were also excluded from the present study.

2.2. Methods

This was a cross-sectional and descriptive study. All clinical and functional parameters were recorded for analysis. Included study subjects were divided into 2 groups: a control group consisting of healthy people without nasal and sinus diseases, and the AR group consisting of patients who met the selection criteria.

The criteria for a diagnosis of AR were: having one of the symptoms of nasal congestion, nasal itching and sneezing, and a runny nose lasting more than 4 days/week according to the season or occurring when exposed to respiratory allergens (dog or cat fur, pollen, mold, and house dust mites) in the living or working environment [11].

2.2.1. Laboratory Functional Testing

The peak inspiratory and expiratory flows (PIF and PEF) in the nose were measured by using a nasal mask-attached peak-flow meter device (Mediflux, Bry Sur Marne, France). Nasal FENO measurement was performed by using multi-flow exhaled NO (Hypair NO,
Medisoft; B-5503 Sorinnes; Belgium). Nasal FENO measurement was carried out according to the manufacturer’s instructions.

2.2.2. Statistical Analyses

SPSS 22.0 software (Chicago, IL, USA) was used to analyze all the collected data. Categorical variables are presented as numbers or percentages. Continuous parameters are presented as means ± standard deviation (SD). The skewness–kurtosis test measured the normal distribution. The Mann–Whitney U test was used for the comparison of means between groups. The correlation between nasal FENO and quantitative variables with normal distribution was examined by regression analysis. A p-value < 0.05 was considered statistically significant.

3. Results

From January 2018 to December 2019, 100 subjects participated in the study, including 32 healthy people (control group) and 68 patients diagnosed with AR (AR group). The latter met the selection criteria and performed all the required functional tests.

3.1. Clinical and Functional Characteristics Study Subjects

There was no significant difference between the AR group and control group regarding age, gender, height, weight, and BMI (p > 0.05; Table 1). The proportion of AR patients who had symptoms of blocked nose, nasal itching or sneezing, and runny nose was 97%, 100%, and 100%, respectively (Table 1). Peak inspiratory and expiratory volumes in patients with AR were significantly lower than in the control group (p < 0.01 and p < 0.01; Table 1). The mean nasal FENO was considerably higher in the AR group than in the control group (985 ± 232 ppb vs. 229 ± 155 ppb; p < 0.001; Table 1).

Table 1. Clinical and functional characteristics of study subjects.

| Characteristics          | All Study Subjects | Patients with AR | Control Subjects | p*    |
|--------------------------|--------------------|------------------|-----------------|-------|
| Number, subjects (%)     | 100 (100.0)        | 68 (68.0)        | 32 (32.0)       | -     |
| Age, years               | 14 ± 3 (6–17)      | 14 ± 3 (6–17)    | 13 ± 4 (6–17)   | >0.05 |
| Sex, male/female         | 1.5                | 1.6              | 1.4             | >0.05 |
| Height, cm               | 135 ± 33 (99–169)  | 134 ± 35 (99–169)| 136 ± 31 (105–167)| >0.05 |
| Weight, kg               | 37 ± 19 (18–57)    | 37 ± 18 (18–55)  | 38 ± 19 (19–57) | >0.05 |
| BMI, kg/m^2              | 16.8 ± 3.3         | 16.7 ± 3.4       | 16.9 ± 3.2      | >0.05 |
| Symptoms of AR           |                    |                  |                 |       |
| Blocked nose, %          | NA                 | 97.0             | 0.0             | NA    |
| Itching and sneezing, %  | NA                 | 100.0            | 0.0             | NA    |
| Running nose, %          | NA                 | 100.0            | 0.0             | NA    |

Nasal peak flow

| Peak inspiratory flow, L/min | 72 ± 22 | 67 ± 14 | 98 ± 26 | <0.01 |
| Peak expiratory flow, L/min  | 107 ± 23 | 93 ± 24 | 124 ± 22 | <0.01 |

Nasal FENO, ppb

| 618 ± 395 (124–1385) | 985 ± 232 (526–1385) | 229 ± 65 (152–299) | <0.001 |

p*: different between AR group and control group; AR: allergic rhinitis; BMI: body mass index; FENO: fractional exhaled nitric oxide; L: liter; ppb: parts per billion; NA: not applicable.
3.2. Correlation between Nasal FENO and the Anthropometric Characteristics of the Control Subjects and Clinical Symptoms in Patients with AR

There was no significant correlation between nasal FENO and the anthropometric characteristics of the control subjects participating in the present study (N = 32; Table 2). Nasal FENO had a significant mild to moderate correlation with clinical symptoms of AR, including blocked nose, itching or sneezing, and runny nose (R = 0.356, 0.679 and 0.587; p < 0.001, 0.0001 and 0.001, respectively; N = 68; Table 2).

Table 2. Correlation between nasal FENO and the anthropometric characteristics of the control subjects and with clinical symptoms in patients with AR.

| Correlation | Anthropometric Parameters (Control Subjects; N = 32) | Symptoms of AR (AR Patients; N = 68) |
|-------------|-----------------------------------------------------|-------------------------------------|
| Nasal FENO  | Age                    | Sex | Height | Weight | BMI | Blocked Nose | Itching or Sneezing | Runny Nose |
|             | 0.098                  | 0.325 | 0.094  | 0.082  | 0.076 | 0.356 | 0.679 | 0.587 |
| P           | 0.124                  | 0.079 | 0.141  | 0.325  | 0.0328 | 0.001 | 0.0001 | 0.001 |

AR: allergic rhinitis; BMI: body mass index; FENO: fractional exhaled nitric oxide.

3.3. Correlation between Nasal FENO and Nasal Peak Flow of Study Subjects

There was no significant correlation between nasal FENO and inspiratory and expiratory peak flow in subjects without AR (control subjects; Table 3). There was a significant and negative linear correlation between nasal FENO and peak inspiratory flow (R = −0.462; p = 0.0012; Table 3, Figure 1a) and peak expiratory flow (R = −0.378; p = 0.0016; Table 3, Figure 1b).

Table 3. Correlation between nasal FENO and nasal peak flow of study subjects.

| Correlation | Control Subjects (N = 32) | AR Patients (N = 68) |
|-------------|---------------------------|---------------------|
| Nasal FENO  | Peak Inspiratory Flow | Peak Expiratory Flow | Peak Inspiratory Flow | Peak Expiratory Flow |
| R           | 0.095 | 0.074 | −0.462 | −0.378 |
| P           | 0.324 | 0.417 | 0.0012 | 0.0016 |

AR: allergic rhinitis; FENO: fractional exhaled nitric oxide.

![Figure 1](image-url)  
(a) Correlation between nasal FENO and peak inspiratory flow in patients with AR. (b) Correlation between nasal FENO and peak expiratory flow in patients with AR. AR: allergic rhinitis; FENO: fractional exhaled nitric oxide.
3.4. Cut-Off of Nasal FENO in the Diagnosis of AR in Children

The cut-off of nasal FENO in positive diagnoses of AR is presented in Figure 2 and Table 4 (N = 100). The results of ROC curve analysis showed that the cut-off of FENO with the highest Youden index was equivalent to the most significant area under the ROC curve of 794 ppb and had a specificity and sensitivity of 96.7% and 92.6%, respectively. (Figure 2, Table 4).

![Figure 2. ROC curve of the nasal FENO cut-off for the diagnosis of AR. AR: allergic rhinitis; FENO: fractional exhaled nitric oxide.](image)

| Nasal FENO Cut-Off (ppb) | Sensitivity (%) | Specificity (%) | Youden Index |
|--------------------------|----------------|----------------|--------------|
| 732                      | 94.2           | 77.3           | 172.189      |
| 738                      | 94.2           | 81.2           | 176.213      |
| 740                      | 94.2           | 83.3           | 178.431      |
| 744                      | 94.2           | 86.1           | 180.346      |
| 749                      | 94.2           | 89.0           | 183.890      |
| 754                      | 93.7           | 93.2           | 187.465      |
| 760                      | 92.4           | 93.1           | 186.767      |
| 794                      | 92.6           | 96.7           | 189.234      |
| 863                      | 91.7           | 95.6           | 188.673      |
| 899                      | 91.4           | 95.6           | 188.348      |
| 905                      | 90.2           | 95.6           | 187.560      |
| 916                      | 89.8           | 95.6           | 186.134      |
| 938                      | 88.2           | 95.6           | 185.778      |
| 945                      | 88.1           | 95.6           | 184.657      |

Table 4. Cut-off nasal FENO with corresponding AR diagnosis sensitivity and specificity.
4. Discussion

The results of our study demonstrated that: (1) Nasal FENO did not depend on anthropometric characteristics or nasal peak inspiratory or expiratory flows in children without AR; (2) there was a correlation between nasal FENO and clinical symptoms, nasal peak inspiratory, and expiratory flows in children with AR; and (3) the cut-off nasal FENO for a diagnosis of AR with the highest specificity and sensitivity was ≥794 ppb.

In healthy people, FENO concentrations in the nose are often much higher than in the lower respiratory tract (300–800 ppb vs. 5–25 ppb). In the rhino-sinusal area, the paranasal sinuses are a vital source of nasal FENO production. Previously, Lundberg et al. [8] described that after perforation of the maxillary sinus, the continuous synthesis of NO at a very high concentration was detected. However, Hood et al. [12] showed that only NO concentrations measured in the nasal cavity came from the sinuses by diffusion due to the NO concentration difference between the nose and sinuses, but it was also produced in the nasal cavity. In the present study, the level of nasal FENO in children without AR symptoms was varied from 152 to 298 ppb (Table 1). This result is also consistent with the manufacturer’s recommendation that the expected value of nasal FENO in children is less than 300 ppb.

The present study showed that, in control children, the level of nasal FENO was not correlated with anthropometric characteristics such as age, gender, height, weight, and BMI (Table 2). Thus, this is a prominent advantage of nasal FENO as a biomarker because it can be used to diagnose various pathological conditions of the nose regardless of demographic features. It might also be similar to bronchial FENO because a previous study also showed that bronchial FENO had no significant correlation with demographic characteristics [13]. However, the recommended cut-off of the normal value of nasal FENO has been established based on a large population that is representative and takes the age into account [14]. The present study only used a control group with a small sample size to determine the nasal FENO value in healthy children compared with AR children.

Because of the short half-life of NO in gas form, indirect methods were previously used to measure the NO concentration in the body during the humoral phase, based on the measurement of NO metabolism products such as nitrate and nitrite, or using immunohistochemistry techniques to determine NOS activity. In contrast to NO produced in tissue or the blood, exhaled NO in the airways is more stable, allowing us to measure it directly [15–17]. Various techniques have been used to measure exhaled NO concentration, and the most commonly used is the chemiluminescence method. This method is highly sensitive, and exhaled NO can be detected at levels as low as parts per trillion. A new NO analysis method based on the electroluminescence technique has been developed and used in clinical practice (Figure 3) [3,18]. This technique has been shown to have high accuracy and good correlation with other methods, and has the advantage of being small compared with fixed routine chemiluminescence analyzers.

The present study results showed that nasal FENO in children with AR was significantly higher than in children without AR (Table 1). Especially in patients with AR, there was a significant correlation between nasal FENO and clinical symptoms (Table 2). In addition, the results also showed that there was a negative and significant correlation between nasal FENO and nasal peak flows (Table 3 and Figure 1a,b). Obviously, exhaled NO concentration is inversely proportional to the airflow rate. FENO measured in healthy subjects with a flow rate of 50 mL/s had a bronchial FENO level of 5–20 ppb, whereas alveolar FENO (CANO) measured at a flow rate of 150–350 mL/s had a concentration of FENO less than 5 ppb [19]. In the present study, nasal FENO was measured with a HypairNO device by the aspirating method with a constant flow over time. However, the application of nasal NO measurement in subjects with AR is relatively complex because some authors have shown that nasal FENO could be changed after allergen exposure. Definitely, Ragab et al. [12] reported that nasal FENO, but not oral FENO, was significantly increased in patients with seasonal AR during the pollen season. However, Palm et al. [13] reported no change in nasal NO concentration in patients with AR. It is noteworthy that
in almost all studies where comorbid sinus disease was excluded, patients with AR had higher nasal NO concentrations compared with healthy subjects. This suggests that there are probably two opposed levels that can determine nasal FENO in patients with RA: firstly, NO gas released from the allergic inflammatory nasal mucosa may be increased although the nasal mucosa are swollen at the same time due to the process of inflammation; secondly, the swollen nasal mucosa might lead to blocked nostrils (ostia) and reduce the flow of NO going out of the nasal cavity, where nasal FENO will be measured.

The present study’s results showed that the nasal FENO cut-off for a positive diagnosis of AR of 794 ppb was the best diagnostic value (Figure 2, Table 4). The results of ROC curve analysis demonstrated that the cut-off of FENO with the highest Youden index was equivalent to the most significant area under the ROC curve of 794 ppb and had a specificity and sensitivity of 96.7% and 92.6%, respectively (Figure 2 and Table 4). However, the sample size of the present study is not large enough to define the nasal FENO cut-off of a large-scale representative population and for subjects with AR associated with other rhino-sinus comorbidities. This issue is also a main limitation of the present study. Therefore, it is necessary to conduct more studies on nasal FENO in subjects with AR for having reference values in the future.

5. Conclusions
Nasal FENO is a potential biomarker in the diagnosis of allergic rhinitis. The measure of nasal FENO is a simple, low-cost, and non-invasive technique. In addition to the use of nasal FENO in the management of patients with allergic rhinitis, nasal FENO might be used for screening patients with sinusitis, nasal polyps, primary ciliary dyskinesia, and Covid-19 infection. Hence, more studies in patients with these conditions are needed in clinical practice to clarify the role of exhaled NO as a relevant biomarker of non-infectious or viral inflammation.
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