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Fractional Exhaled Nitric Oxide: Comparison Between Portable Devices and Correlation With Sputum Eosinophils

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This study was performed to compare the 2 different portable devices measuring fractional exhaled nitric oxide (FeNO) and to see the correlation between FeNO and induced sputum eosinophil count (ISE). Forty consecutive subjects clinically suspected to have asthma underwent FeNO measurement by NIOX-MINO® and NObreath® concurrently. All also had induced sputum analysis, methacholine provocation test or bronchodilator response test, and spin prick test. Agreement between the 2 devices was evaluated. The correlation between FeNO and ISE was assessed, as well as the cut-off level of FeNO to identify ISE ≥ 3%. The intraclass correlation coefficient (ICC) between FeNO levels measured by NIOX-MINO® ($FeNO_{NIOX-MINO}$) and NObreath® ($FeNO_{NObreath}$) was 0.972 with 95% confidence interval of 0.948-0.985. The 95% limits of agreement were -28.9 to 19.9 ppb. The correlation coefficient between ISE and $FeNO_{NIOX-MINO}$ was 0.733 ($P<0.001$), and 0.751 between ISE and $FeNO_{NObreath}$ ($P<0.001$). The ROC curve found that the $FeNO_{NIOXMINO}$ of 37.5 ppb and the $FeNO_{NObreath}$ of 36.5 ppb identified ISE ≥ 3% with 90% sensitivity and 81% specificity. Age, sex, body mass index, smoking history, atopy, and the presence of asthma did not affect the FeNO level and its correlation with ISE. The NIOX-MINO® and NObreath® agree with each other to a high degree. Both devices showed close correlation with ISE with similar cut-off value in identifying ISE ≥ 3%.

Key Words: Asthma; nitric oxide; electrochemical technique; eosinophils; sputum

INTRODUCTION

Since the discovery of nitric oxide in exhaled breath of humans in 1991,[1] evidence has been built up that its level is related to eosinophilic airway inflammation.[2] The level of fractional exhaled nitric oxide (FeNO) is recognized to be useful in diagnosing asthma, predicting steroid responsiveness and monitoring treatment adherence.[3,4] Being easy and noninvasive, FeNO measurement can be of good use in daily practice.

The American Thoracic Society and European Respiratory Society (ATS/ERS) recommendations in FeNO measurement are based on the chemiluminescence-based analyzers,[5] which are bulky and expensive. Electrochemical sensor technology was developed and shown to be comparable to chemiluminescence in measuring FeNO.[6] Recently developed portable devices using electrochemical sensors are smaller and cheaper, thus are more feasible to use in primary care facilities. NIOX-MINO® (Aerocrine AB, Solna, Sweden) is the first device using electrochemical sensor approved by US Food and Drug Administration (FDA) and is now used in practice as well as in research. NObreath® (Bedfont, Rochester, UK) is a newer device using electrochemical sensors.

Several studies verified the compatibility between devices using chemiluminescence analyzer and those using electrochemical sensors.[7-11] To our knowledge, there are 2 studies that directly compared NIOX-MINO® and NObreath®.[12,13] However, the relationship of FeNO measured by these devices with sputum eosinophilia was not evaluated in these studies.

To evaluate the ability to represent eosinophilic airway inflammation as well as the accuracy of the devices, we directly compared NIOX-MINO® and NObreath® in patients with symptoms suggestive of asthma, and performed correlation analysis between FeNO and induced sputum eosinophil count (ISE).

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MATERIALS AND METHODS

Study subjects

Data was prospectively collected in patients suspected to have asthma by history and physical examination on their first visit to the asthma clinic of a tertiary university center. They underwent FeNO measurement by the 2 devices (NIOX-MINO® and NObreath®), pulmonary function test, methacholine provocation test or bronchodilator test, induced sputum analysis and skin prick test. The study was approved by the Institutional Review Board of Samsung Medical Center. All subjects were fully informed of the study protocol and gave written informed consent.

The tests were performed in the following order; FeNO measurement by NIOX-MINO®, FeNO measurement by NObreath®, methacholine provocation test or bronchodilator response test, sputum induction and skin prick test. The rationale of this order is based on the evidence that spirometric maneuvers but not FeNO measurements reduce FeNO levels.[14-17]

FeNO measurement

FeNO measurement was performed according to the ATS/ERS recommendations[5] under the direction of an experienced technician. Subjects were asked to avoid food intake, exercise and smoking within 1 hour before the test. They seated without a nose clip, inhaled to total lung capacity and then exhale at a constant flow rate of 50 mL/s guided by an eye level indicator. They practiced maintaining a constant flow while exhaling into the mouthpiece with the indicator not connected to the device until the technician decides they are capable of doing the actual test. The measurement was done first with the NIOX-MINO®, which decides the acceptability of the test by itself. The first accepted value was recorded. Then the FeNO level was measured by the NObreath®. Because the NObreath® does not determine the acceptability of the test, the first value technician decides as acceptable was recorded. The number of attempts was limited to 3. Both devices were maintained according to the manufacturers' guidelines.

Bronchial provocation and bronchodilator response tests

Methacholine bronchial provocation test was performed according to the ATS guidelines as described previously.[18] Airway hyperresponsiveness was defined by positive methacholine provocation test; fall in forced expiratory volume in one second (FEV1) from baseline of ≥20% with methacholine doses of 16 mg/mL or less. In 3 patients in whom the baseline FEV1 was less than 70% of predicted value, bronchodilator test was performed instead of the methacholine bronchial provocation test.

Sputum induction and analysis

Induced sputum examination was performed as described previously.[18] The specimen was considered adequate if more than 300 non-squamous cells could be counted and if squamous cell count was less than 70% of total cell count.

Skin prick tests

The inhalant allergen skin prick test was performed to determine atopic status. Major inhalant allergens were evaluated, including *Dermatophagoides pteronyssinus*, *Dermatophagoides farinae*, cockroach, grass mix, tree mix, mugwort, ragweed, *Alternaria* spp., *Aspergillus fumigatus, penicillium*, cat and dog. Normal saline was used as negative control and histamine as positive control. Atopy was defined if there are positive results (median wheal size ≥3 mm, larger than size of histamine, and median flare size ≥10 mm) to one or more allergen.

Statistical analysis

Data were presented as median with interquartile range as they did not have normal distribution. All statistical analysis was performed using the SPSS 21.0 (SPSS Inc., Chicago, IL, USA). For comparison between NIOX-MINO® and NObreath®, intraclass correlation coefficient (ICC) was calculated. Bland-Altman plot was used to assess the inter-device agreement. The adequacy of the sample size was verified.[19]

Multiple linear regression analysis was performed to see the effect of possible confounding factors on FeNO suggested by previous studies.[3,5,20] Spearman's correlation coefficient was used to assess the relationship between FeNO measured by each device and ISE. The receiver-operating characteristic (ROC) curve was constructed to determine the level of FeNO measured by each device that best identified ISE ≥ 3%. The results were considered to be significant with the P values ≤0.05

RESULTS

Forty five consecutive patients successfully underwent FeNO measurement by both NIOX-MINO® and NObreath® during the 3 months period from January to April 2014. Five were excluded from this study because they failed to provide adequate sputum samples. The baseline characteristics of the 40 enrolled patients are presented in Table. They aged from 17 to 73 years old. Among them, 26 (65%) were female and 6 (15%) were smokers. The reasons for suspecting asthma were dyspnea, chronic and subacute cough, and previous clinical diagnosis of asthma which was asymptomatic at the time of the study. There were 3 patients using inhaled corticosteroid (ICS) and 3 patients using leukotriene antagonist. They stopped the medications for 2 weeks before the tests. Continuation of nasal corticosteroid was allowed in 3 patients. Among the patients, 11 reported the history of allergic rhinitis and 9 had chronic rhinosinusitis. Asthma was diagnosed in 24 (60%) patients and nonasthmatic eosinophilic bronchitis (NAEB) in 4 (10%) patients. The range of FeNO was 9 to 203 ppb and 9 to 242 ppb, when measured by NIOX-MINO® and by NObreath®, respectively.

The FeNO levels measured by NIOX-MINO® ($FeNO_{NIOX-MINO}$) and by NObreath® ($FeNO_{NObreath}$) were closely correlated with the intraclass correlation coefficient of 0.972 (95% CI, 0.948-0.985; $P<0.001$). The Bland-Altman plot showed the mean difference ($FeNO_{NIOX-MINO}$ minus $FeNO_{NObreath}$) of -4.5 ppb, with 95% limits of agreement from -28.9 to 19.9 ppb (Fig. 1).

Multiple linear regression analysis found no significant association of age, sex, body mass index, smoking history, and atopy with FeNO level measured by both devices. There were close correlations between FeNO level by each device and ISE ($r=0.733$, $P<0.001$ between $FeNO_{NIOX-MINO}$ and ISE; $r=0.751$, $P<0.001$ between $FeNO_{NObreath}$ and ISE). The ROC curves showed that $FeNO_{NIOX-MINO}$ of 37.5 ppb (90% sensitivity and 81% specificity) and $FeNO_{NObreath}$ of 36.5 ppb (90% sensitivity and 81% specificity) identified ISE ≥ 3% (Fig. 2).

Table. Patient Characteristics (N=40)

Characteristics	Values
Age, median years (IQR)	53.0 (32.0-59.0)
Male/Female, n (%)	14 (35)/26 (65)
Smoking history, n (%)	
Non-smoker	26 (65)
Ex-smoker	8 (20)
Current smoker	6 (15)
Reason for suspecting asthma, n (%)	
Dyspnea	12 (30)
Chronic cough*	20 (50)
Subacute cough*	4 (10)
Previous diagnosis of asthma	4 (10)
Final diagnosis, n (%)	
Asthma	24 (60)
Unexplained cough	5 (12.5)
NAEB	4 (10)
Postinfectious cough	3 (7.5)
UACS	2 (5)
COPD	1 (2.5)
GERD	1 (2.5)
$FeNO_{NIOX-MINO}$, median ppb (IQR)	39.5 (19.0-82.8)
$FeNO_{NObreath}$, median ppb (IQR)	39.0 (21.0-93.5)
ISE, median % (IQR)	1.83 (0.08-17.25)

*Chronic cough is defined as cough persisting more than 8 weeks, subacute cough is defined as cough persisting more than 4 weeks, but less than 8 weeks at the time of initial presentation.
IQR, interquartile range; NAEB, nonasthmatic eosinophilic bronchitis; UACS, upper airway cough syndrome; COPD, chronic obstructive pulmonary disease; GERD, Gastroesophageal reflux diseases; FeNO, fractional exhaled nitric oxide; ISE, induced sputum eosinophil.

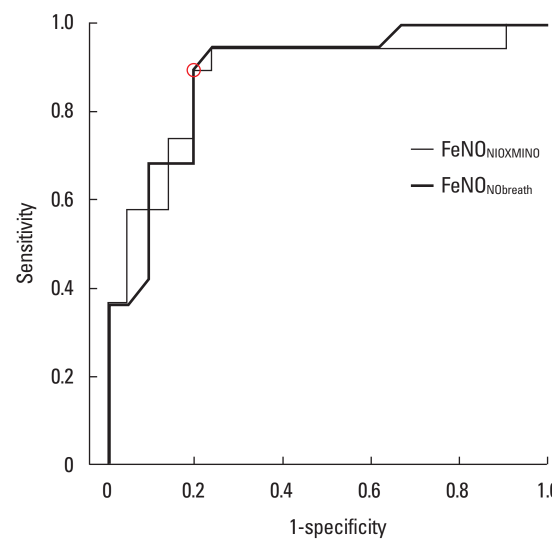

Fig. 2. ROC curve of $FeNO_{NIOX-MINO}$ and $FeNO_{NObreath}$ to identify ISE≥3%. Area under curve is 0.877 and 0.886, respectively for $FeNO_{NIOX-MINO}$ and $FeNO_{NObreath}$. The circle indicates the closest point to the top left-hand corner, which corresponds to 37.5 ppb of $FeNO_{NIOX-MINO}$ and 36.5 ppb of $FeNO_{NObreath}$.

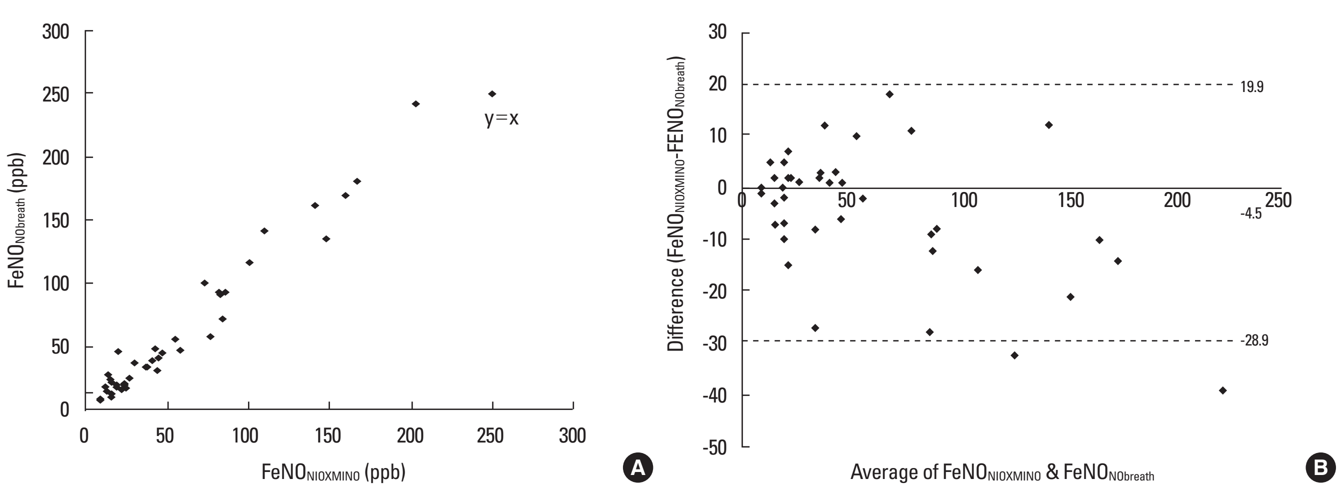

Fig. 1. (A) Correlation between fractional exhaled nitric oxide levels measured by NObreath® and NIOX-MINO®. (B) Bland-Altman plot shows the agreement between NIOX-MINO® and NObreath®.

DISCUSSION

Our study shows that the levels of FeNO measured by NIOX-MINO® and NObreath® agree with each other and that they are strongly correlated to ISE. This is the first study comparing two electrochemical sensor devices in adult patients suspected to have asthma. It is also the first to assess the correlation between FeNO levels measured by electrochemical sensor devices and ISE.

The 2 devices are different from each other in many aspects. NIOX-MINO® only analyzes samples with acceptable exhaling flow, while NObreath® also accepts samples by poor exhaling maneuvers. Instead, NObreath® allows multiple tests in a patient, therefore enables multiple trials in case of poor maneuver. This resulted in poorer repeatability using NObreath® in a study in asthmatic children.[13] The authors of the study recommended at least 3 blows when using NObreath®.[13] This indicates the need for an experienced technician when using NObreath®. In the present study, we allowed 1 or 2 extra blows when the technician decides that the first respiratory maneuver was inappropriate. NIOX-MINO® does not need calibration, but should be replaced every 3,000 tests. NObreath® should be calibrated regularly. NIOX-MINO® device has a 3-year shelf-life, whereas NObreath® is claimed to be semi-permanent. NIOX-MINO® is 230 mm-tall and weighs 800 g, and NObreath® is 152 mm-tall and weighs 400 g.

There was a trend toward higher FeNO measured by NObreath® than by NIOX-MINO® in this study. Antus *et al.*[12] reported similar findings in which NObreath® gave higher FeNO levels compared to NIOX-MINO® with mean difference of 4.2 ppb in 18 healthy adults. On the contrary, a study in 109 children showed FeNO levels measured by NIOX-MINO® being higher than those by NObreath.[13] The mean difference of FeNO level in the latter study was 7.8 ppb with 95% limits of agreement from -11.55 to 27.52 ppb. Other studies found higher FeNO levels measured by NIOX-MINO® compared to various chemiluminescence analyzer devices.[7-9,11] NObreath® was shown to give similar or lower FeNO levels compared to standard machines.[10] The conflicting data shows the distinction between direct and indirect comparison.

The intraclass correlation coefficient (ICC) is a statistical description of agreement between different variables in the same group. It is different from other correlation measures in that it treats the data as groups, not as pairs. The high ICC in this study might partly be attributable to the heterogeneity of the study subjects,[21] in which the FeNO ranged from 9 to 242. Despite high ICC, the Bland-Altman plot gave rather wide range of the limits of agreement compared to other studies. Considering that Bland and Altman suggested the limits of agreement to assess the interchangeability of measurement methods,[22] the result of this study should be interpreted that the devices agree with each other in high degree, but are not interchangeable. Thus in practice, clinicians are free to measure FeNO level by either of the devices, but are recommended to repeat the measurement in a patient with the same device. The choice is a matter of preference.

The correlation between FeNO and ISE has been reported variably. The correlation coefficients ranged from 0.094 (n=81, P=0.406) in healthy nonasthmatic adults,[23] to 0.35 (n=25, P=0.09) in asthmatic children,[24] to 0.48 (n=35, P=0.003) in atopic asthmatic adults,[25] to 0.493 (n=21, $P<0.05$) in adults with NAEB, to 0.576 (n=14, $P<0.05$) in adults with asthma,[26] to 0.59 (n=566, $P<0.001$) in adults with stable asthma,[2] to 0.62 (n=78, $P<0.0001$) in adults with mild to moderate asthma using ICS.[27] The correlation seems to be present only in patients with asthma or NAEB, and 70% of our study population had these diseases. The ROC curves were also concordant with previous studies. The study by Berry *et al.*[2] showed that FeNO measured at flow rate of 50 mL/s detects sputum eosinophilia (ISE $\geq$ 3%) at 36 ppb with a sensitivity and specificity of 78% and 72%, respectively. Oh *et al.*[26] found FeNO level of 31.7 ppb detecting NAEB with a sensitivity of 86% and specificity of 76%.

The adequacy of induced sputum in this study was determined by the squamous cell percentage less than 70% of total cell count. Although many researchers studying induced sputum allows up to 30% squamous cells for adequate sputum sample, there is lack of evidence of appropriate cutoff value of squamous cell contamination. In this study, only 19 (47%) patients provided induced sputum containing less than 30% squamous cell. Recent study by Kim *et al.*[23] reported 19.8% of the induced sputum were inadequate using the 30% cutoff value. We believe the more generous criterion allows more samples to be analyzed without sacrificing the accuracy of the test. A study regarding this issue is in preparation.

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