Exhaled nitric oxide in stable chronic obstructive pulmonary disease

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Abstract:
STUDY OBJECTIVE: The objective of the study was to test the hypothesis that fraction of exhaled nitric oxide (FENO) is elevated in nonsmoking subjects with stable chronic obstructive pulmonary disease (COPD) and compare it with the results in patients with asthma and a control population.

DESIGN: Cross-sectional study.

MATERIALS AND METHODS: Pulmonology Clinic at a University Hospital. Twenty-five control subjects, 25 steroid naïve asthmatics and 14 COPD patients were studied. All the patients were nonsmokers and stable at the time of the study. All subjects completed a questionnaire and underwent spirometry. Exhaled nitric oxide was measured online by chemiluminescence, using single-breath technique.

RESULTS: All the study subjects were males. Subjects with stable COPD had significantly higher values of FENO than controls (56.54±28.01 vs 22.00±6.69; P=0.0001) but lower than the subjects with asthma (56.54±28.01 vs 84.78±39.32; P=0.0285). The FENO values in COPD subjects were inversely related to the FEV₁/FVC ratio. There was a significant overlap between the FENO values in COPD and the control subjects.

CONCLUSION: There is a significant elevation in FENO in patients with stable COPD, but the elevation is less than in asthmatic subjects. Its value in clinical practice may be limited by the significant overlap with control subjects.

Key words:
Asthma, chronic obstructive pulmonary disease, exhaled nitric oxide, stable

Nitric oxide (NO) is a molecular gas, which, in the respiratory system, regulates vascular and bronchial tone leading to dilatation, facilitates the beating of the cilia and acts as a neurotransmitter.[1-3] Although NO is formed by different mechanisms and by various types of cells, the larger central airways is believed to be the main source of exhaled NO (eNO) in humans.[4-5] It was first demonstrated in 1991 that NO could be detected in the exhaled gas of animals and humans in the range of three to 20 parts per billion (ppb), by using chemiluminescence analysis.[6] The measurement of fraction of exhaled nitric oxide (FENO) is, however, affected by a number of factors and varies in disease and health. Although the measurement simple, noninvasive and highly reproducible, it is necessary to pay special attention to the technique. The recommendations by the American Thoracic Society (ATS) and the European Respiratory Society (ERS) have standardized the procedure for the offline and online measurements of eNO,[7] thus making the figures comparable between different research centers. Furthermore, the approval of a portable FENO monitoring device by the United States Food and Drug Administration in 2003 may increase the clinical availability and use of this technology.[8] The measurement of exhaled nitric oxide may, therefore, be an integral component of the care of patients with various respiratory conditions.

Nitric oxide plays an important role as an inflammatory mediator in the airways. The fraction of eNO has been used in asthma to establish the correct diagnosis in steroid naïve patients,[9,10] predict favorable response to corticosteroids,[11,12] titrate anti-inflammatory medications,[13,14] predict impending asthma exacerbation[15,16] and monitor adherence to medications.[17,18] A recent trial suggested that care guided by FENO measurements may achieve similar asthma control at lower steroid doses, as compared to guideline-based management.[19] Thus FENO, which is easily measured, is fast being included in the diagnosis and management of asthma. On the other hand, there are only a few studies describing the levels of FENO in patients with COPD and some of the published reports are conflicting in their conclusions. Also, while some studies reported an increase in the values in patients with stable COPD, others have shown reduced or unchanged values.[20-27] Only one of the studies included patients with asthma as a comparator group.[21]

Recent studies indicate the following:

1. Patients with COPD may respond differently to treatment, depending on the levels of FENO.[28]
2. Increased values of FENO may signify exacerbations.[29]
3. Levels correlate with disease severity.[23]
Assessing FENO may, therefore, play an important role in the evaluation, management and prediction of outcome in patients with COPD, as it has done in the case of asthma patients.

This study was, therefore, conducted to compare FENO levels between former smokers with stable COPD; nonsmoking, steroid-naive asthmatics; and, healthy nonsmoking volunteers.

Materials and Methods

This cross sectional observational study was conducted at the departments of Physiology and Medicine, at the King Khalid University Hospital, King Saud University, Riyadh, Saudi Arabia, from September 2006 to August 2007. Written informed consent was obtained from all the patients and the project was approved by the College of Medicine Ethics Review board.

Seventy five individuals were studied. A total of 64 individuals, who fulfilled the selection criteria were selected for the study - 25 patients with bronchial asthma, 14 patients with COPD and 25 normal controls. Patients classified as COPD had airway obstruction with a forced expiratory volume in the first second (FEV₁) of <80% and FEV₁/FVC ratio of <70%, an FEV₁ increase of <200 ml and 15% of baseline figures after bronchodilator. In addition, patients should have stopped smoking for at least one year and have not had exacerbation in the two months prior to the study. Patients with known atopy or positive skin tests to common allergens were excluded from this group. Patients with significant sputum and peripheral blood eosinophilia were also excluded. Patients were classified as asthmatics, if they had history of physician-diagnosed asthma, demonstrated airway obstruction with an increase of FEV₁ of >15% baseline after bronchodilator and had no history of smoking. Measurement of eNO was done at least 12 hours after the use of β2-agonists in patients on this medication. Similarly, all patients with other systemic diseases such as renal, hepatic, thrombotic and collagen vascular diseases were excluded. Chest radiograph was carried out to exclude other respiratory diseases.

The control group included healthy individuals who were non smokers, without any history of thoracic cage or spinal deformities, respiratory diseases or childhood asthma, allergic rhinitis or atopy. They were matched for age, height, weight, body mass index (BMI) and occupation.

The following studies were performed:

**Ventilatory function parameters**

Forced expiratory volume in 1 s (FEV₁), forced vital capacity (FVC), FEV₁/FVC, peak expiratory flow (PEF), FEF_{25}, FEF_{50} and FEF_{75} were measured by the Vitallograph (ALPHA, Ireland). All recordings were made in sitting position. At least three readings were obtained and the best of the three was taken as the final result.

**Exhaled NO measurements**

FENO measurements were performed according to the present recommendations of the American Thoracic Society,[1] using a NOX EVA 4000 chemiluminescence analyzer (SERES-FRANCE) with a sensitivity of 1 part per billion (ppb).

Using online visual monitoring, the subjects were asked to inhale from residual volume to total lung capacity (TLC). Then, the subjects performed a slow expiratory vital capacity maneuver, with a constant standardized expiratory flow rate of 0.05 L/sec (±10%), resulting in an expiration time of about 20 s, into a Teflon cylinder connected to 3-mm Teflon tubing, without clipping the nose.

To exclude nasal NO contamination, a small expiratory resistance of 1 to 2 cm H₂O was applied. The subjects inspired from atmospheric air and expired in restricted-breath configuration set up.

The expiratory flow rate was measured by a pneumotachograph of data acquisition system BIOPAC MP-100 (Biopac Systems Inc, USA). Plateau levels of FENO against time were determined and expressed as parts per billion (ppb).

Mean exhaled NO concentrations were determined between 5 and 15 s, after the start of the expiration. Three successive recordings at 1 min intervals were made, and the mean was used in the analysis. Nitric oxide concentrations were calibrated two to three times per week, using a standard NO calibration gas.

**Statistical analysis**

The data was analyzed using the computer software program, Statistical Package for Social Sciences (SPSS Version11). Data was expressed as mean ± SD for continuous variables and as percentages for categorical variables. The test applied for statistical analysis was Student’s t-test. A P value of 0.05 was taken as statistically significant and all tests were two tailed. Linear regression analysis was performed between FEV₁/FVC ratio as the dependent variable, and FENO levels in healthy subjects, asthmatics and COPD patients as independent variable.

**Results**

Table 1 gives the clinical characteristics and body composition of control, COPD and asthmatics. The asthmatic patients were younger and had significantly higher body mass index (BMI) than the controls and COPD subjects. The FEV₁, in liters for healthy, COPD and asthmatic subjects were as follows: 3.64 ± 0.57, 1.75 ± 0.61 and 2.52 ± 0.94 respectively. The FVC in liters for healthy, COPD and asthmatic subjects were: 4.28 ± 0.67, 2.65 ± 0.79 and 3.19 ± 0.96 respectively. The FEV₁/FVC ratios for healthy, COPD and asthmatic subjects were: 84.75 ± 4.20, 60.69 ± 7.77, and 78.67 ± 10.15 respectively. FENO levels were significantly higher in both asthmatic (84.78 ± 39.32) and COPD (56.54 ± 28.01) subjects, than in healthy subjects (22.0 ± 6.69, P = 0.0001). The values of FENO measured in parts per billion (ppb) were higher significantly among the asthmatic subjects, as compared to the COPD subjects, the difference being (84.78 ± 39.32 vs 56.54 ± 28.01 P = 0.0285). The results are presented in Figures 1–3 and also Tables 2-4.

**Table 1: Clinical characteristics and body composition of control and COPD patients**

|                     | Control n = 25 | COPD n = 14 | Asthma n = 25 |
|---------------------|----------------|-------------|---------------|
| **Age (years)**     | 51.78 ± 6.83   | 54.70 ± 5.87| 37.92 ± 14.22|
| **Height (cm)**     | 171.78 ± 4.32  | 165.20 ± 12.40| 175.25 ± 8.14|
| **Weight (kg)**     | 87.73 ± 20.91  | 91.33 ± 35.50| 82.51 ± 15.89|
| **Body mass index** | 29.54 ± 6.01   | 26.11 ± 3.44| 33.38 ± 7.97*|

*P < 0.05 vs asthmatics
Furthermore, Figures 1-3 give the linear regression analysis between the FEV1/FVC ratio and FENO levels in healthy, asthmatic and COPD subjects.

Among patients with COPD, there was a negative correlation between the FEV1/FVC ratio and the level of FENO. The values of FENO among COPD, asthmatic and control ranged between 14-105, 42-175, and 3-40 ppb respectively. While there was no overlap between the FENO figures among asthmatic and healthy subjects, a number of subjects with COPD had figures within the range found among healthy subjects.

**Discussion**

The most significant finding in this study is that FENO is elevated in nonsmoking, nonatopic patients with stable COPD and that this elevation is less than that seen in patients with asthma. Furthermore, there is a significant overlap in the values in subjects with COPD and normal subjects. There is also a correlation between the level of FENO and the FEV1/FVC ratio in COPD subjects, but not in asthmatics or normal subjects. Normal FENO levels have not been defined for our population; but, in the selection of controls, all possible confounding
In consonance with our study, Brindicci et al. reported that perfusion mismatch and, therefore, a powerful potential simple indicator of the degree of lung damage (ventilation-divided-perfusion mismatch) and, therefore, a powerful potential simple indicator of the degree of lung damage (ventilation-divided-perfusion mismatch). This implies that the level of FENO may be an early and independent marker of lung damage.

Factors that may contribute to the elevation in FENO levels include chronic inflammation and remodeling, resulting in increased peripheral resistance. Although the levels of FENO are elevated in both asthma and COPD, there is a high level expression of inducible NO synthase (iNOS) present in sputum macrophages alveolar walls, small airway epithelium and vascular smooth muscle of these patients. This may result in an increased production of NO and NO-related species in the lung periphery. Although the levels of FENO are elevated in both asthma and COPD, the levels are much higher in the latter group. There is data to suggest that the factors leading to the elevation of FENO in COPD are different from those in asthma. As an example, although there is a relationship between the use of corticosteroids and levels of FENO in asthma, no such relationship exists in COPD. There is, however, a relationship between the levels of FENO in COPD and measures of lung function abnormality, as shown by Ansarin et al. Exhaled NO inversely correlated with FeNO, DlCO and SaO2, and was positively correlated with the residual lung volume/total lung capacity ratio in their study. It is also known that the progression of COPD from GOLD stage 0 to 4 is most strongly associated with thickening of the wall of small airways by a repair or remodeling process. Early authors suggest an alternative explanation for the elevation of FENO levels in patients with diffuse pulmonary damage, secondary to years of tobacco smoking. Nitric oxide is a highly reactive chemical, with a short half-life within the body. At the alveolar level, NO combines rapidly with reduced haemoglobin and is, therefore, scavenged by pulmonary capillary blood. In the presence of altered ventilation-perfusion ratio, this scavenging will take place less efficiently, leading to higher levels of exhaled NO.

In COPD, there is accumulation of inflammatory mucous exudates in the lumen and infiltration of the small airway wall by inflammatory cells, as the disease progresses. There is a high level expression of inducible NO synthase (iNOS) present in sputum macrophages alveolar walls, small airway epithelium and vascular smooth muscle of these patients. This may result in an increased production of NO and NO-related species in the lung periphery. Although the levels of FENO are elevated in both asthma and COPD, the levels are much higher in the latter group. There is data to suggest that the factors leading to the elevation of FENO in COPD are different from those in asthma. As an example, although there is a relationship between the use of corticosteroids and levels of FENO in asthma, no such relationship exists in COPD. There is, however, a relationship between the levels of FENO in COPD and measures of lung function abnormality, as shown by Ansarin et al. Exhaled NO inversely correlated with FeNO, DlCO and SaO2, and was positively correlated with the residual lung volume/total lung capacity ratio in their study. It is also known that the progression of COPD from GOLD stage 0 to 4 is most strongly associated with thickening of the wall of small airways by a repair or remodeling process. Early authors suggest an alternative explanation for the elevation of FENO levels in patients with diffuse pulmonary damage, secondary to years of tobacco smoking. Nitric oxide is a highly reactive chemical, with a short half-life within the body. At the alveolar level, NO combines rapidly with reduced haemoglobin and is, therefore, scavenged by pulmonary capillary blood. In the presence of altered ventilation-perfusion ratio, this scavenging will take place less efficiently, leading to higher levels of exhaled NO.

In conclusion, this study corroborates other studies that FENO is increased in stable, nonsmoking COPD but that the levels are lower than in asthma. The considerable overlap between the values seen in COPD and controls precludes its use as a diagnostic tool. Nevertheless, the development of new hand-held analyzers will make the measurement more readily available, make larger comparative studies possible and hence make possible application in day to day practice.
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