Research Article

Fractional exhaled nitric oxide is correlated with pulmonary function in patients with stable chronic obstructive pulmonary disease

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Abstract

Chronic obstructive pulmonary disease (COPD) is characterized by progressive airflow obstruction and airway inflammation. Nitric oxide (NO) is a known marker for airway inflammation in several lung diseases. Measurement of fractional exhaled nitric oxide (FeNO) is an easy and non-invasive method for assessing airway inflammation. Whether FeNO levels are associated with lung functions remain to be established. We evaluated airway inflammation and its correlations with pulmonary functions in stable COPD patients. Fifty stable COPD patient and 50 control males aged 44 to 83 years old participated in this study. All subjects underwent FeNO testing and spirometry. The Global Initiative for Chronic Obstructive Lung Disease (GOLD) 2015 Guidelines were used to classify the severity of airflow limitation in COPD patients. The proportion of patient with COPD 1, 2, 3, and 4 were 28%, 38%, 32%, and 2%, respectively. Mean FeNO levels were significantly higher in COPD patients than in controls (35.3 ± 8.1 vs. 11.4 ± 4.4 ppb, $p<0.001$). FeNO levels significantly increased in proportion to severity of COPD. Moreover, FeNO level in COPD patients was inversely correlated with pulmonary function as assessed by %predicted FVC ($r = -0.527$), FEV_1 ($r = -0.770$), MEF ($r = -0.693$) and PEF ($r = -0.540$) ($p<0.001$). The present study suggests that FeNO level has clinical relevance, since it increases with increasing severity of COPD and is negatively associated with pulmonary function.

Keywords: Chronic obstructive pulmonary disease, Airway inflammation, Lung function

Introduction

Chronic obstructive pulmonary disease (COPD) is a common preventable and treatable disease. It is characterized by persistent airflow limitation which is usually progressive and associated with enhanced chronic inflammation in the airways and lungs. [1] It is accompanied by systemic manifestations that have a severe impact on the morbidity and mortality of patients. [2] Nitric oxide (NO) is a biological mediator in humans. NO produced by the lungs has been implicated in the pathophysiology of lung diseases [3] since it plays an important role in regulating airway and vascular function and is a marker of airway inflammation and can be also detected in various pulmonary diseases, such as asthma and COPD. [4] It is generated by three isoforms of NO synthases (NOS) including neuronal NOS (nNOS), endothelial NOS (eNOS) and inducible NOS (iNOS), each with a different expression and pathophysiologic role in the airway. [5] nNOS and eNOS are constitutively expressed and calcium dependent. These are found in the airway epithelium, where they produce picomolar concentrations of NO. iNOS is expressed in vivo in the bronchial epithelial cells in humans and produces nanomolar concentrations of NO, which remains stable in the gaseous phase and can be assayed. Moreover, its activity increases during certain inflammatory processes. [6] In smokers, airway inflammation in COPD is probably due to cigarette smoking-induced release of inflammatory cytokines and oxygen free radicals resulting in increased iNOS. NO is synthesized by the conversion of the amino acid L-arginine to L-citrulline by iNOS by residential and inflammatory cells in the airways or alveoli. NO has a significant role in regulating airways. [5] Interestingly, the usefulness of fractional exhaled nitric oxide (FeNO) in monitoring therapeutic intervention and its clinical relevance in COPD is still uncertain. Measuring FeNO level is a quantitative, noninvasive, simple, and safe method for assessing airway inflammation. Further, it is useful as a complementary tool for evaluating airway diseases, especially asthma. [3] There are conflicting data regarding the value of FeNO levels in assessing COPD, and therefore measurement is less relevant in clinical practice. [7] Some studies have reported increased [8, 9] or similar [10, 11] FeNO levels in stable COPD patients compared to healthy subjects. Severe COPD patients show lower exhaled NO levels than ex-smokers and mild/moderate COPD patients. [12] Increased exhaled NO levels have been reported in hospitalized patients during an exacerbation of COPD. [13] Some studies suggested that FeNO is a good surrogate marker of eosinophilic inflammation in COPD patients with exacerbation. [14, 15] Previous study has demonstrated that the blood eosinophil count is a likely biomarker that can predict positive relationship with FeNO values and pulmonary function parameters. [16] There is conflicting evidence regarding the association between FeNO levels and lung function. Previous studies reported that FeNO levels are either inversely related [17-19] or not related to forced vital capacity (FVC) or forced expired volume in one second (FEV_1) or FEV_1/FVC. [20] Moreover, FeNO levels are negatively related to diffusion capacity of carbon monoxide (DLCO) and oxygen saturation (SaO_2) and positively related to the residual lung volume/total lung capacity ratio. [17]

Therefore, the aims of our study were to evaluate the pulmonary function and FeNO levels, and to assess the correlation between FeNO levels and pulmonary function in COPD patients. We hypothesized that patients with stable COPD may show the changes in airway inflammation that

increase following the severity of COPD, as compared to healthy subjects.

Material and Method

Subjects: The study population consisted of two groups: 50 male patients with stable COPD (45 to 83 years old) recruited from Srinagarind Hospital and Khon Kaen Hospital, and 50 healthy males (44 to 77 years old) from Khon Kaen Province, Thailand.

COPD patients recruited to the group were diagnosed by specialists using the criteria of the Global Initiative for Chronic Obstructive Lung Disease (GOLD) Guidelines. [1] COPD patients with history of atopy, asthma or other pulmonary diseases, upper respiratory tract infection, chest infection, COPD exacerbation during the period three month prior the beginning of the study, hypertension, diabetes or engaging in a regular physical exercise program in the period six months prior to the beginning of the study were excluded. Healthy subjects were non-smokers and had no history of disease. All subjects with history of other diseases associated with high FeNO (e.g. viral respiratory tract infections, systemic lupus erythematosus, liver cirrhosis, acute lung allograft rejection and post-transplant bronchiolitis obliterans) or history of other diseases associated with low FeNO (e.g. cystic fibrosis, HIV infection and pulmonary hypertension) were excluded as well. Patients were instructed not to use any bronchodilator or inhaled steroid 12 h before performing the lung function test and FeNO measurement. Also, all subjects were advised not to consume any nitrite-rich foods before the study visits. Patients with COPD were treated with short-acting beta-2 agonists (SABA), long-acting beta-2 agonists (LABA), inhaled corticosteroids (ICS), long-acting muscarinic antagonists (LAMA) and methylxanthine.

Informed consent was obtained from all subjects after explanation of all the measurements that were to be done. The methods used in this study were reviewed and approved by the Ethics Committee for Human Research (HE 571287).

Pulmonary function test: Pulmonary functions including FVC, FEV_1, and mid-expiratory flow (MEF) and peak expiratory flow (PEF) were measured using a Vitalograph Pneumotrac (Vitalograph, Ireland). The measurements used for analysis were taken from the best of three recordings made in the sitting position with a nose clip in place according to the ATS/ERS Standardization of Lung Function Testing. [21] Values were expressed as liters as well as percentages of the predicted normal values (%predicted) in Thai population. [22] Spirometry tests were performed on the same day as the measurement of FeNO.

FeNO measurements: FeNO was measured according to the ATS/ERS guidelines using Quark NObreath (COSMED Srl, ITALY) [23], and is expressed as parts per billion (ppb). FeNO was measured by asking subjects to inhale to total lung capacity, and to exhale through the mouthpiece, keeping the ball in the flow indicator in the middle of the white band in the center of the tube, at a constant flow of 50 ml/s or 12 seconds of exhalation. Calibration of the analyzer was automatically performed by the software. All subjects were asked to refrain from eating, drinking, and strenuous exercise for two hours prior to FeNO measurement.

Statistical analysis: Data were expressed as mean ± standard deviation (SD). Unpaired t-test was used to compare COPD patients and controls for demographic characteristics and pulmonary function. Two-sample Wilcoxon rank-sum (MannWhitney) test was used when data deviated from normality. Statistical analyses were performed using a STATA version 12.0 (StataCorp, College Station, TX). The correlation between FeNO level and pulmonary function was measured using a linear regression analysis. A P value less than 0.05 was considered to be statistically significant.

Results

The baseline characteristics of the patients and controls are shown in Table 1. COPD patients had a smoking history of 40.2 ± 23.0 pack-years while control subjects were non-smokers. There were 14, 19, 16 and 1 patients with GOLD stage 1, 2, 3 and 4, respectively. Number of COPD patients treated with SABA, LABA, LABA+ICS, ICS, LAMA and methylxanthine are shown in Table 1. Weight and body mass index (BMI) were significantly lower in COPD patients compared to controls (55.1 ± 10.9 vs. 59.8 ± 7.6 kg, p<0.05 and 21.0 ± 3.5 vs. 23.1 ± 1.9 kg/m2, respectively, p<0.001) (Table 1). There were no statistically significant differences in age, systolic blood pressure (SP), diastolic blood pressure (DP) and mean arterial pressure (MAP) among the two groups.

Pulmonary function results are reported in Table 2. Pulmonary function in COPD patients was significantly lower compared to controls. FeNO levels in COPD patients were 3.1 times higher than that of healthy subjects (35.3 ± 8.1 vs. 11.4 ± 4.4 ppb, p<0.001) **(Figure 1)**. Moreover, FeNO levels in COPD patients significantly increased with severity of the disease **(Figure 2)**. FeNO levels in each stage of COPD were as follows: GOLD stage 1 28.8 ± 2.2 ppb (with a range of 26 - 33 ppb), GOLD stage 2 31.9 ± 4.5 ppb (with a range of 26 - 40 ppb), GOLD stage 3 43.8 ± 5.4 ppb (with range of 35 - 55 ppb) and GOLD stage 4 56.0 ppb. The differences in FeNO levels between patients in different GOLD stages were statistically significant. In addition, the difference in FeNO levels between COPD patients and controls was also statistically significant.

Moreover, there were significantly negative correlations between FeNO levels and %predicted FVC (r = -0.527), FEV_1 (r = -0.770), MEF (r = -0.693) and PEF (r = -0.540) in stable COPD patients (p<0.001), but not in controls **(Figure 3).**

Table 1 Baseline characteristics of COPD patients and controls

Characteristics	Controls (n = 50)	COPD (n = 50)
Age (years)	64.1 ± 8.4	66.5 ± 9.5
Weight (kg)	59.8 ± 7.6	55.1 ± 10.9*
Height (cm)	160.8 ± 7.5	161.8 ± 6.6
BMI (kg/m2)	23.1 ± 1.9	21.0 ± 3.5***
SP (mm Hg)	121.6 ± 10.7	123.8 ± 11.3
DP (mm Hg)	72.7 ± 10.3	75.0 ± 8.9
MAP (mm Hg)	89.0 ± 9.0	91.2 ± 7.8
Smoking history (pack-years)	-	40.2 ± 23.0
SABA	-	30
LABA	-	9
LAMA	-	3
LABA+ICS	-	9
ICS	-	46
Methylxanthine	-	33

BMI, body mass index; SP, systolic blood pressure; DP, diastolic blood pressure; MAP, mean arterial pressure; SABA, short-acting beta-2 agonists; LABA, long-acting beta-2 agonists; ICS, inhaled corticosteroids; LAMA, long-acting muscarinic antagonists. Values are mean ± SD. *p<0.05 and ***p<0.001 COPD vs. controls.

Table 2 Pulmonary functions and FeNO levels in COPD patients and controls

Parameters	Controls (n = 50)	COPD (n = 50)
FVC (L)	3.51 ± 0.67	2.38 ± 0.71***
FVC (%predicted)	96.70 ± 10.36	71.91 ± 16.86***
FEV_1 (L)	2.96 ± 0.66	1.53 ± 0.57***
FEV_1 (%predicted)	98.78 ± 11.21	63.09 ± 20.12***
FEV_1/FVC (%predicted)	102.10 ± 7.34	87.46 ± 19.22***
MEF (L/s)	3.60 ± 1.02	1.06 ± 0.63***
MEF (%predicted)	96.34 ± 17.19	39.85 ± 22.51***
PEF (L/s)	7.72 ± 1.60	3.90 ± 1.85***
PEF (%predicted)	91.80 ± 10.60	54.91 ± 24.95***
FeNO (ppb)	11.4 ± 4.4	35.3 ± 8.1***

FVC, forced vital capacity; FEV_1, forced expired volume in one second; MEF, mid-expiratory flow; PEF, peak expiratory flow; FeNO, fractional exhaled nitric oxide. Values are mean ± SD. ***p<0.001 COPD vs. controls.

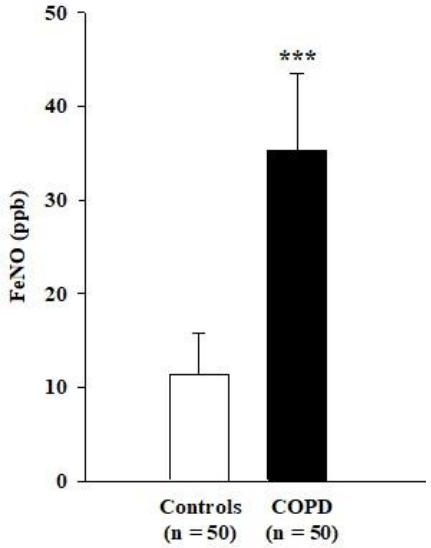

Figure 1 FeNO levels in COPD patients and controls.

***p<0.001 vs. controls

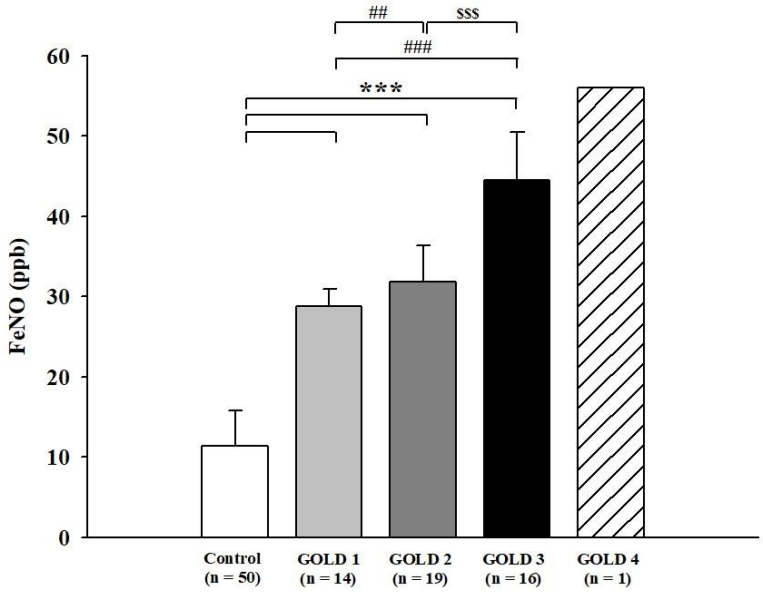

Figure 2 FeNO levels in each stage of COPD.

***p<0.001 vs. controls, ##p<0.01 and ###p<0.001 vs. GOLD I, $$$p<0.001 vs. GOLD II

GOLD IV had only one patient, so it cannot compare with other groups.

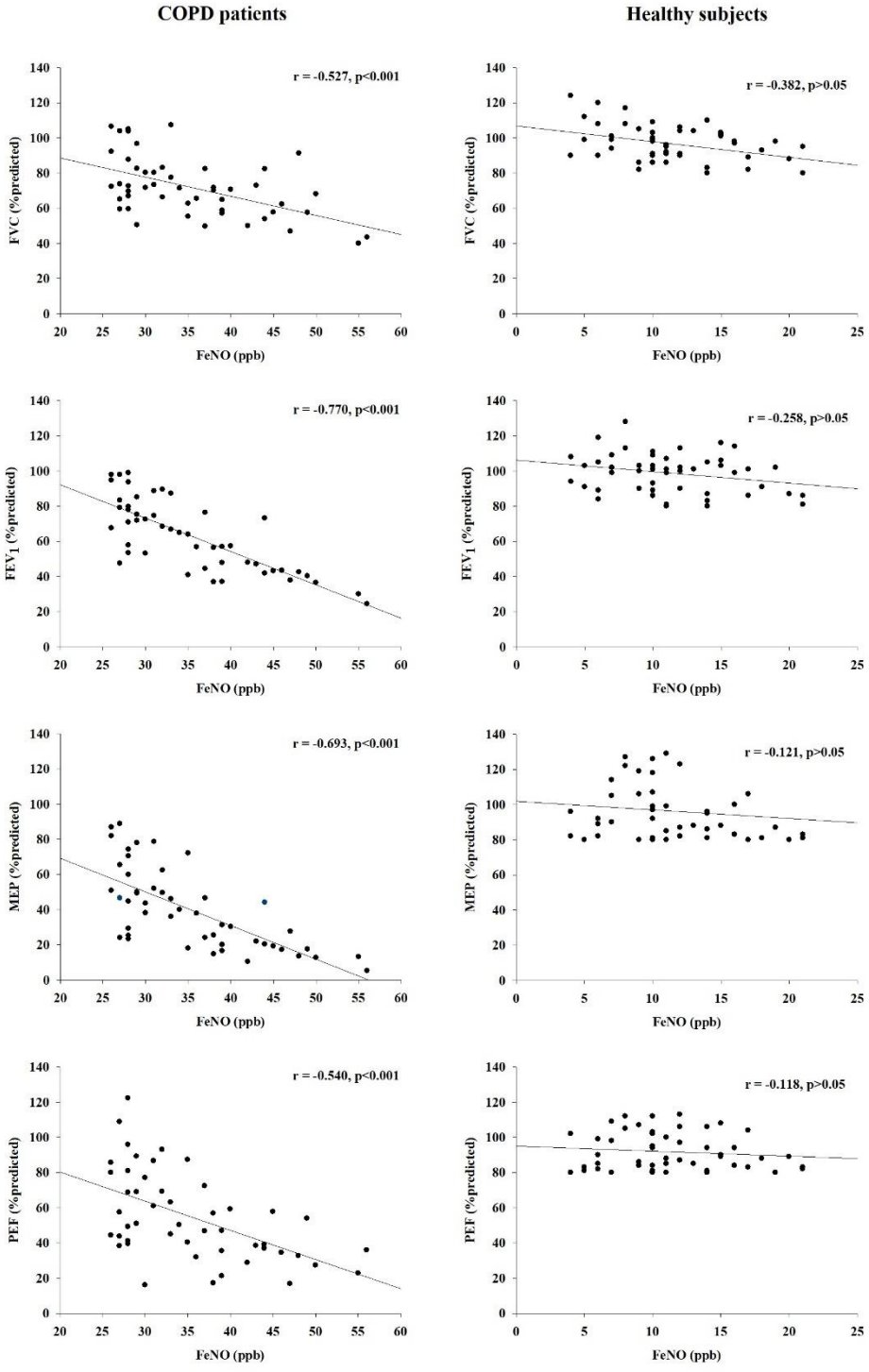

Figure 3 Linear regression analysis showing relationships between %predicted of FVC, FEV_1, MEF and PEF and FeNO levels in COPD patients and healthy subjects.

Discussion

These results reveal that FeNO levels in patients with stable COPD were higher compared to controls, and FeNO levels increased with increasing severity of COPD. Moreover, there was also a significantly negative correlation between levels of FeNO and pulmonary function, i.e. % predicted FVC, FEV_1, MEF and PEF in patients with COPD, but not in controls.

Our findings showed decreases in weight and BMI in COPD patients compared to controls. The reduced weight and, hence, BMI were likely due to muscle wasting caused by many factors such as hypoxemia, malnutrition, oxidative stress, and systemic inflammation. [24] Decreased appetite caused by hypoxia-induced expression of leptin and detraining results in muscle wasting. [25] Additionally, extra work required for breathing and the presence of systemic inflammation leading to increased basal metabolism together with a decreased appetite results in a negative nutrition balance and ultimately weight loss. [26, 27]

iNOS is expressed in epithelial cells in response to pro-inflammatory cytokines and oxidants, probably via activation of the transcription factor nuclear factor kappa B (NF-KB). [28] Previous studies have detected an accumulation of NO and NO-related species in the periphery of the lungs in COPD and asthma patients. [29] NOS is expressed in the following cell types: arterial and venous endothelial cells, epithelial cells, macrophages, mast cells, neutrophils, eosinophils, non-adrenergic non-cholinergic nerves, fibroblasts, smooth muscle cells and platelets. [30] High levels of iNOS expression have been reported in macrophages, alveolar walls, small airway epithelium and vascular smooth muscles of COPD patients. [31] Moreover, there was significant correlation between exhaled NO with the percentage of neutrophils and sputum IL-8 in patients with COPD. [18]

The finding that FeNO levels in COPD patients were higher compared to those of controls is consistent with several previous studies. [17-19] However, other studies found no difference in exhaled NO levels between COPD patients and healthy subjects. [10, 11] The reason for the discrepancy between our study and other studies reporting no difference may be due to a small number of participants and/or inappropriate comparison. [10, 11] In contrast, the present study was carried out in 50 controls and 50 COPD patients. Increased FeNO levels in COPD patients are probably due to structural abnormalities of tobacco-induced lung damage [27] as a consequence of iNOS induction by superoxide anion from mediators in cigarettes. [31]

In COPD patients, mucus resulting from inflammation exudes into the lumen and inflammatory cells accumulate in the small airway wall as the disease progresses [32] and consequently, greater FeNO levels are observed. This observation suggests that exhaled NO levels may be used for assessing disease development in COPD. In fact, the authors of previous study suggest that measurements of NO may be useful in monitoring inflammation and progression of COPD, and the response to anti-inflammatory treatment. [33] In addition, the recent study demonstrated that FeNO levels were associated with the severity of COPD and allergic airway inflammation. [34] In the other hand, current study reported that FeNO test is not routinely recommended in international or national clinical guidelines for patients with COPD alone. [35] FeNO is a biomarker which has the potential to be used as a complementary value for differentiating COPD with concurrent diagnosis of asthma from COPD-

only. [36] However, the FeNO levels in COPD patients remains unclear. This discrepancy may in part be explained by the heterogeneous nature of the disease, with multiple factors influencing FeNO levels. [2]

The present study also found inverse correlations between FVC, FEV_1, MEF and PEF, with FeNO levels in COPD patients. The finding that FEV_1 is negatively correlated with FeNO levels is in agreement with a previous study. [17] This study also reported a correlation between FeNO levels and DLCO and SaO_2, and a positive correlation with the residual lung volume/total lung capacity ratio in COPD patients. It is 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. [37] Several studies suggest that elevated FeNO levels in COPD patients may also be used as an indicator of increased spirometric response to corticosteroid treatment. [38, 39] Moreover, a previous study suggested that glucocorticoids inhibit the induction of iNOS in epithelial cells and reduce the elevated exhaled NO in patients with inflammatory airway diseases. [28] However, our data showed no differences in FeNO levels between COPD patients with or without ICS.

In conclusion, the present study demonstrates increased FeNO levels in stable COPD patients. FeNO levels are positively correlated with the severity of COPD, and are inversely correlated with pulmonary function in patients with stable COPD. The current study also suggests that FeNO measurement may be useful for assessing the severity of COPD and treatment in COPD patients. Although this study was carefully prepared, we are still aware of its limitations and shortcomings. First, the study was conducted in small number of participants leading to a relatively small sample size to compare the FeNO levels between COPD patients and healthy subjects. Second, our study did not head to evaluate the role of monitoring treatment, we could not follow-up serial FeNO levels regularly, simply focused the initial FeNO levels.

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