



Levels of Exhaled Breath Condensate pH and Fractional Exhaled Nitric Oxide in Retired Coal Miners

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Inhaled inorganic dusts, such as coal, can cause inflammation and fibrosis in the lungs, known as pneumoconiosis. Diagnosis of pneumoconiosis depends on morphological changes by radiological findings and functional change by pulmonary function test (PFT). Unfortunately, current diagnostic findings are limited only to lung fibrosis, which is usually irreversibly progressive. Therefore, it is important that research on potential and prospective biomarkers for pneumoconiosis should be conducted prior to initiation of irreversible radiological or functional changes in the lungs. Analytical techniques using exhaled breath condensate (EBC) or exhaled gas are non-invasive methods for detection of various respiratory diseases. The objective of this study is to investigate the relationship between inflammatory biomarkers, such as EBC pH or fractional exhaled nitric oxide (FE_{NO}), and pneumoconiosis among 120 retired coal miners (41 controls and 79 pneumoconiosis patients). Levels of EBC pH and FE_{NO} did not show a statistically significant difference between the pneumoconiosis patient group and pneumoconiosis patients with small opacity classified by International Labor Organization (ILO) classification. The mean concentration of FE_{NO} in the low percentage FEV₁ (< 80%) was lower than that in the high percentage (80% ≤) ($p = 0.023$). The mean concentration of FE_{NO} in current smokers was lower than that in non smokers (never or past smokers) ($p = 0.027$). Although there was no statistical significance, the levels of FE_{NO} in smokers tended to decrease, compared with non smokers, regardless of pneumoconiosis. In conclusion, there was no significant relationship between the level of EBC pH or FE_{NO} and radiological findings or PFT. The effects between exhaled biomarkers and pneumoconiosis progression, such as decreasing PFT and exacerbation of radiological findings, should be monitored.

Key words: Exhaled breath condensate, Fractional exhaled nitric oxide, pH, Lung inflammation, Pneumoconiosis

INTRODUCTION

Among occupational lung diseases, the most prevalent diseases are induced by inhalation of dust, including asbestos, crystalline silica, and coal. Inhalation of these types of dust may result in development of a variety of pulmonary diseases, such as a nodular interstitial lung disease, progressive massive fibrosis (PMF), emphysema, and classical pneumoconiosis, including coal workers pneumoconiosis (CWP) (Schins and Borm, 1999). Exposure to silica and coal mine dust may also result in pulmonary scarring in a pattern that mimics idiopathic pulmonary fibrosis, and in chronic obstructive pulmonary disease

(COPD), including emphysema and chronic bronchitis, which appears indistinguishable from obstructive lung disease caused by exposure to tobacco smoke. Exposure to coal mine and silica dust may therefore result in restrictive, obstructive, or mixed patterns of impairment on pulmonary function testing. Many researchers are aware of the nodular fibrosis pulmonary tissue reactions in response to retained dust; however, they may not realize that these other reactions of the pulmonary parenchyma and airways to dust exist and can result in significant respiratory dysfunction in sensitive individuals. Fibrosis of tissue resulting from these types of dust may invoke functional damage and irreversible change (Cohen *et al.*, 2008). Notably, crystalline silica has been classified as a class I carcinogen by the International Agency for Research on Cancer (IARC, 1997).

Pneumoconiosis is a lung disease caused by inhalation of

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mine dust. Diagnosis of pneumoconiosis depends on morphological changes by radiological findings and functional change by pulmonary function test. Unfortunately, there is no cure for the damage and current diagnostic findings are only limited to fibrosis in the lung, which is usually irreversibly progressive. Once silica threshold has been exceeded, silica-induced pulmonary disease may progress without further exposure to silica. Therefore, it is important that research on potential and prospective biomarkers for pneumoconiosis should be conducted prior to the occurrence of irreversible radiological changes in the lung (Gulumian *et al.*, 2006; Porter *et al.*, 2004). Many researchers have studied biomarkers in pulmonary inflammation resulting from mineral dust (Schins and Borm, 1999).

Detection of inflammatory biomarkers for pulmonary diseases involves invasive techniques, such as blood, bronchoalveolar lavage fluid (BALF), and induced sputum. Because invasiveness sampling procedures to obtain specimens may induce an inflammatory response, non-invasive techniques, such as exhaled breath condensate (EBC) and exhaled air have been developed. Analyses of various biomarkers in exhaled breath have allowed non-invasive monitoring of inflammation and oxidative stress in respiratory tract in inflammatory lung diseases (Grob *et al.*, 2008; Lehtonen *et al.*, 2007; Goldbart *et al.*, 2006; Horvath *et al.*, 2005; Montuschi, 2005; Kharitonov and Barnes, 2002). Studies of reactive oxygen species (ROS) using direct analysis of free radicals in EBC of patients with fibrosis have been reported (Rosias *et al.*, 2006).

pH and fractional exhaled nitric oxide (FE_{NO}) are simple and technically validated biomarkers studied in exhaled air (Grob *et al.*, 2008; Hunt, 2007). The pH of EBC has been found to be lower in many respiratory disorders, including COPD (Kostikas *et al.*, 2002; Borrill *et al.*, 2005), asthma (Grob *et al.*, 2008; Hunt *et al.*, 2000), bronchoectasis (Kostikas *et al.*, 2002), and chronic cough (Niimi *et al.*, 2004) than in normal subjects. Therefore, many researchers have suggested that the pH of EBC could be useful a biomarker for detection of inflammatory acidification in the respiratory tract (Grob *et al.*, 2008; Hunt, 2007). Exhaled NO released from lung cells, including inflammatory cells, epithelial cells, and endothelial cells in the respiratory tract has been known to be an effective biomarker in diagnosis and treatment of lung disorders and correlated with decrease of pulmonary function (Paredi *et al.*, 2002; Maziak *et al.*, 1998). Reported lung disorders associated with the levels of FE_{NO} include asthma (Kharitonov and Barnes, 2006) and COPD (Ferreira *et al.*, 2001). Yet, there have been no reports on EBC in lung disorders that affect Korean patients. The objective of this study was to investigate the relationship between EBC pH or FE_{NO} as inflammatory biomarkers and pneumoconiosis findings obtained from radiological findings identified by the pneumoconiosis review committee and pulmonary function test (PFT).

MATERIALS AND METHODS

Subjects. The study population included 120 retired male miners exposed to coal dust. Data on EBC, urine, chest x-ray, and PFT were obtained from the subjects. Personal information on age, body weight, height, and various personal histories (job, smoking status, and disease) was obtained using a structured questionnaire. The Research Ethics Committee of our institute approved the study protocol, and all participants gave informed written consent.

Collection of EBC. Collection of EBC was performed in accordance with recommended guidelines of the American Thoracic Society (Horvath *et al.*, 2005). Briefly, all participants rinsed their mouths with purified saline prior to collection. Exhaled breath condensate was collected during oral tidal breathing using the Rtube™ EBC collection system (Respiratory Research Inc, USA). All samples were collected for 10 minutes, and nose clips were worn. The condensate was transferred from the collection tube into cryogenic vials and stored at -80°C until assay.

Measurement of EBC pH. The pH of EBC was assayed with a glass microelectrode (Orion 4Star/9802BN electrode, Orion, USA) after gas standardization through bubbling 150 μL of EBC with purified argon (99.99% <) for 8 min, as previously described (Accordino *et al.*, 2008).

Measurement of FE_{NO} . Measurement of exhaled NO was performed as recommended by the American Thoracic Society/European Respiratory Society guidelines (American Thoracic Society, 2005). Briefly, FE_{NO} was measured with an NO analyzer (Sievers 280i, GE Analytical Instruments Inc., USA). While wearing nose clips, participants performed continuous inhalation of nitric oxide free air and exhaled against a positive pressure of 16 mmH_2O for longer than 6 seconds and generated expiratory flow of 50 mL/sec .

Analysis of urinary cotinine. Analysis of urinary cotinine, metabolite of nicotine, was measured by high performance liquid chromatography (HPLC, Agilent 1200 series; Agilent, USA), as previously described (Noriaki *et al.*, 1993). Briefly, 3 mL of the urine samples and standard were added with 1 mL of 2 N sodium hydroxide. After mixing, 2 mL of dichloromethane was added and was centrifuged at 3,000 rpm for 10 min. After evaporation under nitrogen flow, suspended samples and standards with 800 μL of deionized water and 20 μL of suspended sample were injected into the HPLC. Cotinine was separated on a C18 column (150 \times 4.6 mm, 3.5 μ ; Agilent, USA). Detecting wavelength was 260 nm.

PFT. Using a spirometer (Vmax22, SensorMedics, USA), PFT was performed in accordance with recommended

guidelines of the American Thoracic Society/European Respiratory Society Task Force (Brusasco *et al.*, 2005). Forced vital capacity (FVC), which is the volume delivered during an expiration made as forcefully and completely as possible starting from full inspiration, forced expiratory volume in one second (FEV₁), which is the volume delivered in the first second of an FVC maneuver, and FEV₁/FVC ratio (%FEV₁/FVC) were measured and calculated for predicted volume by the regression equation of Morris *et al.* (1971).

$$\text{FVC predicted volume (L)} = 0.148 \times \text{height (in)} - 0.025 \times \text{age (yr)} - 4.241$$

$$\text{FEV}_1 \text{ predicted volume (L)} = 0.092 \times \text{height (in)} - 0.032 \times \text{age (yr)} - 1.26$$

Predicted percentages (%) of FVC (%FVC) and FEV₁ (%FEV₁) were calculated by the following equation:

$$\% \text{ predicted} = \frac{\text{measured volume (L)}}{\text{predicted volume (L)}} \times 100$$

PFT was performed with the patient in the sitting position via the closed circuit method, measuring inhaled and exhaled air during the same test cycle. Tests were conducted until gaining 3 adequate sets of data.

Chest x-ray. Radiological findings for pneumoconiosis were observed on digital chest x-ray (Digital Diagnost, Philips, Netherlands). Diagnosis of pneumoconiosis was identified by the pneumoconiosis review committee of the Korea Worker's Compensation & Welfare Service, and classifications were categorized in accordance with classification of ILO (2002).

Statistical analysis. Data were analyzed using SPSS 14.0 (SPSS, Chicago, IL, USA). General characteristics and PFT data showed normal distribution, whereas FE_{NO} showed log-normal distribution (Kolmogorov-Smirnov test); therefore, data on FE_{NO} was log-transformed for all of the statistical tests, and the results were expressed as the geometric mean (GM) and geometric standard deviation (GSD). EBC pH did not show normal or log-normal distribution; therefore, data on EBC pH was analyzed by nonparametric statistics. A *p*-value of <0.05 (two-tailed) was considered significant for all of the tests.

RESULTS

General characteristics of the study populations.

Number of study subjects classified by the ILO categories of pneumoconiosis (ILO, 2002) was "small opacity" in 62 (51.6%), type I in 44 (36.6%), type II or III in 18 (15.0%), and "large opacity" in 17 (14.2%) (Table 1).

Characteristics of the study populations are shown in Table 2. Average age in the control group (n = 41), the small

Table 1. Numbers of each pneumoconiosis category according to the ILO classification

ILO category*	N	%	Profusion (N)
Control	41	34.2	0/0 (34); 0/1 (7)
Pneumoconiosis	79	65.8	
Small opacity	62	51.6	
I	44	36.6	1/0 (16); 1/1 (19); 1/2 (9)
II, III	18	15.0	2/1 (10); 2/2 (6); 2/3 (1); 3/2 (1)
Large opacity	17	14.2	A (17)

Table 2. General characteristics of the study subjects

	Control (n = 41)	Pneumoconiosis		<i>p</i> -values
		Small opacity (n = 62)	Large opacity (n = 17)	
Age (yrs)	60.9 ± 8.8	64.6 ± 7.5	65.3 ± 6.6	0.042 ^a
BMI (kg/m ²)	24.1 ± 2.3	23.7 ± 2.6	23.1 ± 2.3	0.354 ^a
Exposure period (yrs)	14.4 ± 6.5	18.4 ± 7.7	16.9 ± 7.0	0.025 ^a
FVC (L)	3.50 ± 0.78	3.36 ± 0.69	3.01 ± 0.64	0.063 ^a
FVC, % predicted	87.0 ± 18.6	89.3 ± 19.0	80.7 ± 18.5	0.250 ^a
FEV (L)	2.50 ± 0.67	2.33 ± 0.53	1.95 ± 0.44	0.004 ^a
FEV ₁ , % predicted	86.5 ± 20.1	87.8 ± 19.3	74.2 ± 18.1	0.037 ^a
FEV ₁ /FVC ratio (%)	71.8 ± 12.3	69.6 ± 8.6	65.3 ± 10.1	0.088 ^a
Smoking, N (%)				
Never	7 (17.1)	5 (8.1)	2 (11.8)	0.120 ^b
Past	11 (26.8)	32 (51.6)	7 (41.1)	
Current ^c	23 (56.1)	25 (40.3)	8 (47.1)	

[†]Mean ± SD

^aCalculated by ANOVA test

^bCalculated by χ^2 -test

^cUrinary cotinine levels of self-reported non-smokers (2 cases) and past-smokers (7 cases) were greater than 785 $\mu\text{g/g}$ creatinine (data not showed), which was the lower limit of the confidence interval in self-reported current smokers, the selected cut-off level for identification of current smokers, and they were biochemically classified as current smokers

opacity group ($n = 62$), and the large opacity group ($n = 17$) were 60.9 ± 8.8 , 64.2 ± 7.5 , and 65.3 ± 6.6 years, respectively. Percent of FEV₁ in pneumoconiosis with large opacity ($74.2 \pm 18.1\%$) was lower than those of the control ($86.5 \pm 20.1\%$) and small opacity ($87.8 \pm 19.3\%$) ($p = 0.037$). Levels of BMI, %FVC, %FEV₁, and number of smokers did not show significant statistical differences among the pneumoconiosis groups.

Levels of EBC pH and FE_{NO} according to general characteristics. No significant differences were observed between median of EBC pH and general characteristics, such as age, BMI, exposure period, smoking status, and criteria levels of %FVC, %FEV₁, and %FEV₁/FVC (Table 3).

The mean concentration of FE_{NO} in current smokers was lower than that in non smokers (never or past smokers) ($p = 0.027$) (Fig. 1), and the mean concentration of FE_{NO} in the low percentage FEV₁ ($< 80\%$) was lower than that in the high percentage ($80\% \leq$) ($p = 0.023$). However, no significant differences were observed between the mean concentration of FE_{NO} and other general characteristics, including age, BMI, exposure period, and criteria levels of %FVC and %FEV₁/FVC.

Levels of EBC pH and FE_{NO} according to ILO categories of pneumoconiosis. Levels of EBC pH and FE_{NO} did not show statistical differences among the pneumoconiosis groups (small or large opacity), and the levels of EBC pH

Table 3. Concentrations of EBC pH and FE_{NO} according to general characteristics

Characteristics	N	EBC pH		FE _{NO}		
		AM (Median)	Range	GM (GSD)*	95% CI	
Age (yrs)	-59	36	7.94 (8.11)	5.71~8.51	18.7 (1.4)	16.6~21.1
	60-69	56	7.99 (8.02)	6.42~8.58	19.2 (1.6)	17.0~21.6
	70-	28	7.73 (7.95)	5.41~8.33	23.2 (1.6)	19.3~28.0
			$p = 0.088^a$		$p = 0.090^c$	
BMI (kg/m ²)	< 25	83	7.91 (8.02)	5.41~8.58	19.4 (1.5)	17.7~21.2
	25	37	7.93 (7.97)	5.71~8.50	21.1 (1.6)	18.2~24.5
			$p = 0.689^b$		$p = 0.647^d$	
Exposure period (yrs)	-9	20	8.02 (8.08)	7.60~8.28	18.8 (1.4)	15.8~22.3
	10-19	55	7.96 (8.01)	5.71~8.58	19.7 (1.5)	17.6~22.2
	20-	45	7.82 (7.96)	5.41~8.46	20.6 (1.6)	18.0~23.3
			$p = 0.811^a$		$p = 0.710^c$	
Smoking status	Never	14	7.84 (7.95)	5.76~8.38	24.4 (1.4)	20.0~29.8
	Past	50	8.06 (8.06)	7.18~8.58	21.0 (1.6)	18.6~23.8
	Current	56	7.81 (7.98)	5.41~8.51	18.0 (1.5)	16.1~20.1
			$p = 0.125^a$		$p = 0.027^c$	
%FVC predicted	80 ≤	84	7.91 (8.01)	5.41~8.58	20.1 (1.5)	18.4~22.0
	< 80	36	7.92 (8.01)	5.76~8.51	19.4 (1.6)	16.4~22.9
			$p = 0.705^b$		$p = 0.654^d$	
%FEV ₁ predicted	80 ≤	74	7.94 (8.01)	5.71~8.58	21.3 (1.4)	19.6~23.2
	< 80	46	7.88 (8.00)	5.41~8.51	17.8 (1.6)	15.4~20.6
			$p = 0.998^b$		$p = 0.023^d$	
%FEV ₁ /FVC ratio	70 ≤	66	7.93 (8.06)	5.71~8.58	20.9 (1.5)	19.1~23.0
	< 70	54	7.89 (7.99)	5.41~8.46	18.7 (1.6)	16.4~21.3
			$p = 0.363^b$		$p = 0.153^d$	

*Geometric mean (Geometric standard deviation), 95% confidence interval, Unit: ppb

^aCalculated by Kruskal-Wallis H test

^bCalculated by Mann-Whitney U test

^cCalculated by ANOVA test

^dCalculated by t-test

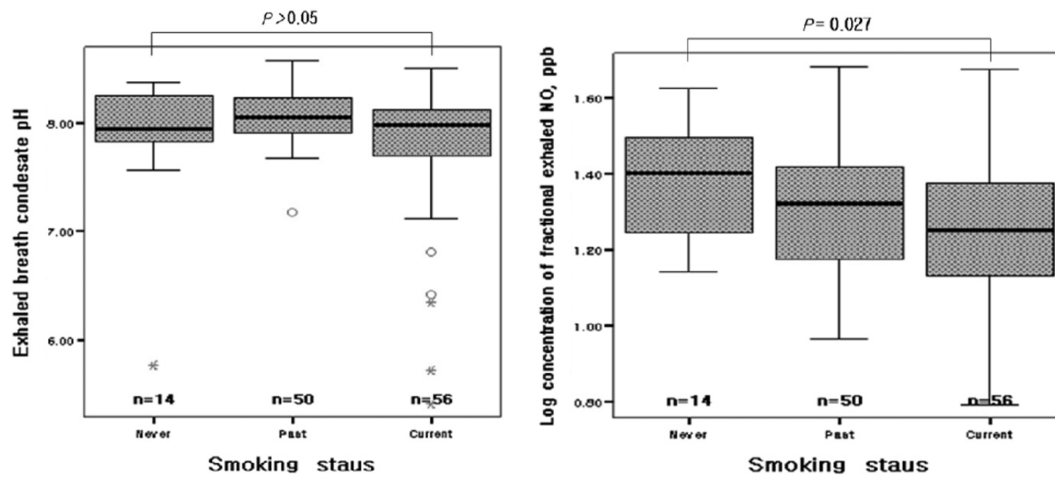


Fig. 1. EBC pH and FE_{NO} according to smoking status.

Table 4. Levels of EBC pH of pneumoconiosis patients

Pneumoconiosis		N	AM ^a	Median	Range	Mean rank	p-values ^b
Opacity	Control	41	7.85	7.93	5.71~8.46	53.8	0.264
	Small	62	7.93	8.02	6.42~8.51	62.8	
	Large	17	8.00	8.03	5.41~8.58	68.3	
ILO Categories ^c	0	41	7.85	7.93	5.71~8.46	47.4	0.223
	I	44	7.92	8.08	5.41~8.58	57.9	
	II or III	18	7.95	7.97	7.57~8.28	48.1	

^aArithmetic mean, arithmetic standard deviation
^bCalculated by Kruskal-Wallis H test
^cSubjects except for patients with large opacity
 ILO Categories (0: 0/0, 0/1; I: 1/0, 1/1, 1/2; II: 2/1, 2/2, 2/3, III: 3/2)

Table 5. Levels of FE_{NO} of pneumoconiosis patients

Pneumoconiosis		N	GM ^a	GSD	95% CI	p-values ^b
Opacity	Control	41	21.1	1.5	18.6~23.9	0.264
	Small	62	20.2	1.6	18.0~22.7	
	Large	17	16.4	1.4	13.6~19.7	
ILO Categories ^c	0	41	21.1	1.5	18.6~23.9	0.847
	I	44	20.0	1.6	17.3~23.2	
	II or III	18	20.8	1.5	17.1~5.2	

^aGeometric mean, geometric standard deviation, unit: ppb
^bCalculated by ANOVA test
^cSubjects except for patients with large opacity
 ILO Categories (0: 0/0, 0/1; I: 1/0, 1/1, 1/2; II: 2/1, 2/2, 2/3, III: 3/2)

and FE_{NO} did not show statistical difference among the groups (ILO category II or III) in pneumoconiosis patients with small opacity (Table 4-5).

Relationship between FE_{NO} levels and associated variables. As shown in Table 6, FE_{NO} was significantly correlated with %FEV1 ($\beta = 0.289, p = 0.001$), smoking status ($\beta = -0.232, p = 0.008$), and BMI ($\beta = 0.227, p = 0.010$) (adjusted R² = 0.141, $p < 0.001$) in the stepwise multiple regression analysis.

DISCUSSION

Toxicity of crystalline silica and coal dust are based on activation of macrophages and lung inflammation. Many researchers have shown concern with regard to crucial mediators of the pulmonary disorder resulting from these mineral dusts (Schins and Borm, 1999). Inhaled dusts or other transitional metals, including iron, copper, and vanadium have been known to induce ROS generated from activated phagocytes in the lung (Becker *et al.*, 1996; Castranova

Table 6. Stepwise multiple regression analysis of log (FE_{NO}) against associated variables

Variables	B ^a	SE	β	p-values
Intercept	0.704	0.179		0.000
BMI	0.017	0.006	0.227	0.010
Smoking status ^b	-0.086	0.032	-0.232	0.008
%FEV ₁ predicted	0.003	0.001	0.289	0.001
R ²			0.162	
Adjusted R ²			0.141	
F			7.485 (<i>p</i> < 0.001)	

^aB: regression coefficients, SE: standard error, β: standardized B

^bDummy variable: yes or no

Exclusion variables: age, work period, pneumoconiosis (dummy variable), %FVC predicted, %FEV₁/FVC ratio, EBC pH, urinary cotinine (log transformed)

et al., 1997; Dalal *et al.*, 1991; Shoemaker *et al.*, 1995; Tourmann and Kaufmann, 1994; Vallyathan, 2004; Wal-laert *et al.*, 1990).

Various harmful materials, including carcinogens and factors of oxidative stress are present in cigarettes (Pryor, 1997). Smoking status has been an important variable in epidemiology studies, especially occupational lung diseases, including pneumoconiosis (Balint *et al.*, 2001; Nowak *et al.*, 2001; Montuschi *et al.*, 2000; Nowak *et al.*, 1996). Self-reports rely on participant's answers, which may be suspect due to hiding of their smoking habits. Cotinine is the major degradation product of nicotine metabolism (Noriaki *et al.*, 1993). Biological cotinine level has been established as a useful biological indicator of actual smoking status. Information on smoking status from face-to-face interviews was compared with laboratory urinary cotinine levels. Urinary cotinine levels of self-reported non-smokers (2 cases) and past-smokers (7 cases) were greater than 785 µg/g creatinine, which was the lower limit of the confidence interval in self-reported current smokers, the selected cut-off level for identification of active smokers. Therefore, they could be biochemically classified as current smokers. Discrepancy was observed between smoking status using self-reported and identification using urinary cotinine. Therefore, simultaneous estimation of biological indicators and self-reports for cigarette smoking were necessary for health surveillance, especially in the status encouraging prohibition of smoking or compensation for the disability of life.

Pneumoconiosis is the most prevalent lung disease showing decrease of pulmonary function (Schins and Borm, 1999). In this study, mean of %FEV₁ of CWP patients with large opacity (74.2 ± 18.1%) was significantly higher than those of the control (86.5 ± 20.12%) and CWP patients with small opacity (87.8 ± 19.3%) (*p* = 0.037).

Similar to biomarkers found in blood and urine, breathing analysis such as EBC, could be a promising non-invasive biomarker for detection of respiratory diseases as well systemic diseases. EBC has been studied in a variety of dis-

eases, including asthma, COPD, cystic fibrosis, allergic rhinitis, and lung cancer (Grob *et al.*, 2008). EBC can be collected by cooling or freezing exhaled air, thereby condensing vapor and aerosolized droplets emerging with the breath (ATS workshop proceeding, 2006).

In this study, measurement of EBC pH was performed after bubbling argon gas for 8 min for removal of CO₂, because measurement of EBC pH after deaeration for removal of CO₂ is a more validated technique (Kostikas *et al.*, 2002; Vaughan *et al.*, 2003). Deaeration caused a significant increase in EBC pH, with a mean change of 2.10 (from 5.91 to 8.01) (results not shown), which is similar to previous reports (Borrill *et al.*, 2006; Niimi *et al.*, 2004). Median pH of EBC in total subjects in this study was 8.01 (range 5.41~8.58), which is similar to previous studies (Paget-Brown *et al.*, 2006; Wells *et al.*, 2005).

Acidification occurs in several respiratory diseases (Borrill *et al.*, 2008; Hunt, 2007; Hovarth *et al.*, 2005). The level of EBC pH is unaffected by variables that include subject age, race, gender, collecting and storage temperature, acute airway obstruction, ammonia in the mouth, saliva pH, hyper- and hypoventilation (Bloemen *et al.*, 2007; Paget-Brown *et al.*, 2006; Borrill *et al.*, 2005; Wells *et al.*, 2005; Vaughan *et al.*, 2003). Acidification of EBC occurs in acidic materials generated from inflammatory cells (Hunt *et al.*, 2002), and gastroesophageal reflux (Effros *et al.*, 2005). Many researchers have suggested that EBC pH is a simple, reproducible, and relevant biomarker of disease (Hunt, 2007; Vaughan *et al.*, 2003). However, in this study, there were no significant differences between EBC pH and measured variables, including general characteristics, criteria levels of pulmonary function test, and radiological findings for pneumoconiosis. Effros *et al.* (2006) reported that EBC pH may not be a reliable marker of airway acidification, because EBC acidification can be affected by the balance of volatile salivary acids and bases. Nicolaou *et al.* (2006) also reported that there were no significant relationships between EBC pH and PFT and bronchitis. Thus, Borrill *et al.* (2008) suggested that more research on the relationship between EBC pH and pulmonary inflammation would be necessary.

FE_{NO} in association with asthma and has been extensively investigated and has been shown to correlate with predominantly eosinophilic airway inflammation and to be reduced by corticosteroid therapy (Kharitonov and Barnes, 2006; Kharitonov *et al.*, 2002). In this study, mean concentrations of non-smokers, non-pneumoconiosis, and non-COPD subjects were 24.4 ppb, 21.1 ppb, and 20.9 ppb, respectively. These results, which are lower than the mean concentration (26 ppb) of the control studied by Girgis *et al.* (2002), are higher than that (17.8 ppb) of the non-smoking control studied by Degano *et al.* (2009).

In this study, we found that FE_{NO} in smokers (18.0 ppb) was lower than in either never-smokers (24.4 ppb) or past-smokers (21.0 ppb) (*p* = 0.027), and that the levels of FE_{NO}

showed a statistical relationship among smoking status ($\beta = -0.232$, $p = 0.008$). These results are in agreement with those of previous studies (Degano *et al.*, 2009; Malinowski *et al.*, 2006; Brindicci *et al.*, 2005). The explanation for these results could be that decreased exhaled NO in smokers is the result of muscular, structural, and metabolic damage resulting from alterations of skeletal muscle, such as oxidative fiber atrophy, increased glycolic capacity, and reduced expression of the constitutive NO synthase (Montes de Oca *et al.*, 2008), or may be due to the effect of tobacco smoking, which down-regulates epithelial NO synthase, and may reflect increased oxidative stress that may consume NO in formation of peroxynitrite (Kharitonov and Barnes, 2002).

Exposure to coal mine dust may result in COPD, including emphysema and chronic bronchitis, that appears indistinguishable from obstructive lung disease. Coal mine and silica dust may therefore result in restrictive, obstructive, or mixed patterns of impairment on pulmonary function testing (Cohen *et al.*, 2008). In this study, no significant differences in FE_{NO} were observed among radiological findings for pneumoconiosis. The mean concentration of FE_{NO} in the low percentage FEV_1 ($< 80\%$) was lower than that in the high percentage ($80\% \leq$) ($p = 0.023$). However, there were no significant differences between the mean concentration of FE_{NO} and criteria levels of $\%FEV_1/FVC$, which is another criteria index for COPD. The explanation for these results may be that radiological findings or decreased PFT are the results of inflammation or fibrosis in the lung; however, FE_{NO} results from the current response of oxidative stress due to inflammation or exposure to hazardous materials. Another possibility may be that PFT is affected by differences in the anatomy of the respiratory tract, such as restriction of bronchus. Conventionally measured FE_{NO} in COPD is less useful, since the levels are usually normal or slightly elevated, except during exacerbations (Kharitonov and Barnes, 2006; Brindicci *et al.*, 2005). New techniques for measurement of exhaled NO have recently been extended by measurements of exhaled NO at different flows (Degano *et al.*, 2009; Condorelli *et al.*, 2007; George *et al.*, 2004; Tsoukias *et al.*, 1998).

In conclusion, no significant relationship was observed between the level of EBC pH and FE_{NO} and radiological findings and PFT. It was necessary to monitor the effects between exhaled biomarkers and pneumoconiosis progression, including decreasing PFT and exacerbation of radiological findings. Future studies will be required for investigation of more specific and sensitive biomarkers that are present in pneumoconiosis patients using new analytical techniques such as proximal and peripheral NO analysis.

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