Original Article

Serum Levels of Interleukin-8 and Tumor Necrosis Factor-alpha in Coal Workers' Pneumoconiosis: One-year Follow-up Study

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Objectives: Various cytokines induced by inhalation of coal dust may mediate inflammation and lead to tissue damage or fibrosis, such as coal workers' pneumoconiosis (CWP).

Methods: To investigate the relevance of serum cytokines in CWP, the levels of serum interleukin-8 (IL-8) and tumor necrosis factor-alpha (TNF-α) as CWP biomarkers in 110 retired coal miners (22 controls and 88 CWP subjects) were related to cross sectional findings and 1-year progressive changes of the pneumoconiosis. Progressive changes of CWP were evaluated by paired comparison of chest radiographs. Analysis by a receiver operating characteristic (ROC) curve assessed the biomarker potential of each cytokine.

Results: The mean serum IL-8 level was significantly higher in CWP compared to controls and IL-8 levels correlated with the degree of CWP. The median serum TNF-α level was significantly higher in subjects with progressive CWP compared to subjects without CWP progression. The area under the ROC curve for IL-8 (0.70) and TNF-α (0.72) for CWP identification and progression, respectively, indicated the biomarker potential of the two cytokines. Serum cutoff values of IL-8 and TNF-α were 11.63 pg/ml (sensitivity 69%; specificity 64%) and 4.52 pg/ml (sensitivity 67%; specificity 79%), respectively.

Conclusion: The results suggest that high levels of serum IL-8 are associated with the presence of CWP and those of serum TNF-α are associated with the progression of CWP.

Key Words: Coal workers' pneumoconiosis, Interleukin-8, Tumor necrosis factor-α, ROC, Follow-up

Introduction

Of the various occupational lung diseases, those induced by inhalation of dusts such as asbestos, crystalline silica, and coal are most prevalent. Inhalation of dusts may cause a variety of lung diseases such as coal workers' pneumoconiosis (CWP), progressive massive fibrosis, chronic alveolitis, and emphysema [1]. Notably, crystalline silica has been classified as a class I carcinogen by the International Agency for Research on Cancer [2]. CWP is a lung disease caused by inhalation of coal dust. Once a silica threshold has been exceeded, silica-induced pulmonary disease may progress without further exposure to silica. Clinical detection of CWP, however, is currently dependent on radiological and lung function abnormalities, which are both late diagnostic tools. Identification of accurate and reliable biomarkers would enable earlier detection before irreversible radiological changes in the lung occur [3,4].

Cytokines influence various biological events such as

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inflammation, metabolic mechanism, cell growth and proliferation, morphogenesis, fibrosis, and homeostasis. Major sources of cytokines in the lung are epithelial cells, endothelial cells, fibroblasts, and inflammatory cells [5]. In previous reports, the relationship between pulmonary inflammation and dusts, and cytokines has been demonstrated for mediators of various toxicological and pathological effects, and several cytokines related with coal dust [6-10]. In one study, the initial concentrations of tumor necrosis factor-alpha (TNF-α) were related to later progression of CWP. Miners who showed abnormally high dust-stimulated release of TNF-α had an increased risk of progression in CWP. TNF-α in pneumoconiosis induced by coal dust was reported to be a powerful tool to estimate individual prognosis of pneumoconiotic disease, even after the end of occupational exposure [11]. Interleukin-8 (IL-8) is a chemokine secreted by a variety of cells types including fibroblasts in response to IL-1 and TNF-α [12]. IL-8 is an important activator and chemoattractant for neutrophils, and has been implicated in a variety of inflammatory diseases [13]. IL-8 is important in the lung inflammation produced by crystalline silica. Both TNF-α and IL-8 were reported to be increased in the supernatant of spontaneous or dust-stimulated monocytes isolated from peripheral blood and in sera of CWP, which did not include progressive CWP [14].

While various studies have addressed the increased production of IL-8 following exposure crystalline silica or coal mine dust in macrophage and fibroblasts, only one human validation study has been published [1]. The available data suggest the importance of IL-8 and TNF-α in CWP. However, little information exists concerning the *in vivo* relevance of predictive discrimination between the levels of cytokines and progression of CWP. To determine the significance of serum cytokines regard to progression of CWP, a longitudinal design is necessary. However, follow-up studies dealing with the prognostic usefulness of cytokines are scarce in Korea.

The present study examined the relationship between serum levels of IL-8 and TNF-α, and CWP findings in retired coal miners with previous cross sectional findings, and examined the relationship between initial levels of same cytokines and 1-year progressive changes of CWP.

Materials and Methods

Study design

Fig. 1 shows the design of the study. A group of 136 male retired coal miners, who were randomly selected from the examinee for diagnosis of pneumoconiosis at Ansan Workers' Compensation Insurance Hospital, Korea Workers' Compensation & Welfare Service, were recruited for follow-up. The study was performed using one-year prospective data and cross-sectional findings. One-year progression of CWP was evaluated by the paired comparison of chest-radiographs made in 2007 and 2008. Chest radiographs in 2008 were measured only in 110 subjects. Ultimately, 110 out of 136 subjects previously screened for serum cytokines (81% of initial subjects) participated in the follow-up.

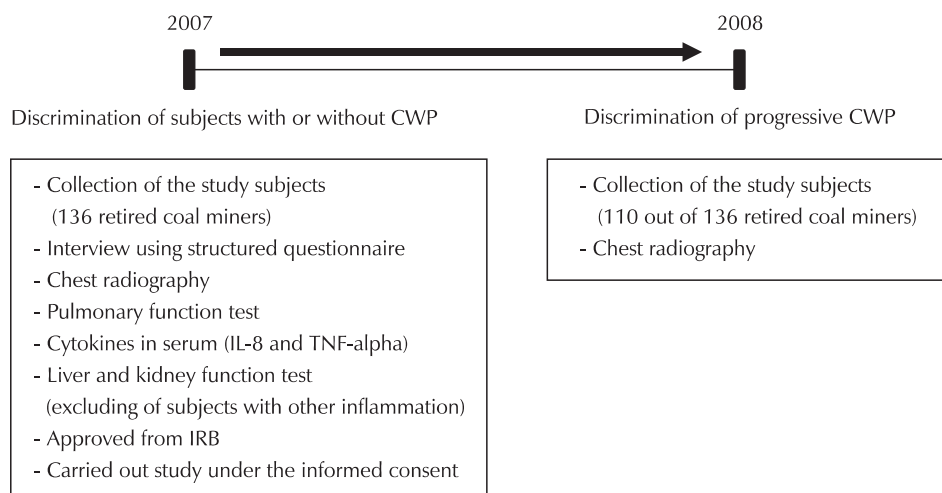Retired coal miners who did not have CWP (ILO clas-

Fig. 1. Design of the study. Serum cytokines (IL-8 and TNF-α) of retired coal miners were related to presence of coal workers pneumoconiosis (CWP) and to progression of CWP. Pulmonary function was determined spirometry data (FVC and FEV$_1$) in 2007. Progression of CWP was evaluated by paired comparison of chest-radiographs made in 2007 and 2008. General characteristics were surveyed from structured questionnaire and interviews.

sification of 0/0 or 0/1) were chosen as controls and CWP patients were identified from physician lists in the pneumoconiosis review committee. CWP patients were divided into two groups according to the presence and the absence of progressive changes on chest radiographs.

Pulmonary function was determined in 2007 by spirometry data that included results of forced vital capacity (FVC) and forced expiratory volume in one second (FEV_1). Pulmonary function test (PFT) and personal information including age, body mass index (BMI), and various personal histories (job and smoking status) were obtained by a structured questionnaire. All subjects provided informed consent and the study was approved by the Research Ethics Committee of our institute (Fig. 1).

Analysis of serum cytokines

Serum was separated from whole blood samples, and stored at –80°C until assay. Analysis of serum cytokines was measured by an EV 3513 cytokine biochip array (Randox Laboratories, Crumlin, U.K.) using sandwich and competitive chemiluminescence immunoassays, as previously described [15,16]. Detection limits of IL-8 and TNF-α were 1.5 pg/ml and 2.6 pg/ml, respectively.

PFT

PFT was measured in accordance with recommended guideline of ATS/ERS Task Force [17] using a Vmax22 spirometer (SensorMedics, San Diego, CA, USA). Measured parameters were FVC (the volume delivered during an expiration made as forcefully and completely as possible starting from full inspiration), FEV_1 (the volume delivered in the first second of an FVC maneuver), and the FEV_1/FVC ($\%FEV_1/FVC$) ratio. The predicted volumes of FVC and FEV_1 were calculated by a by previously reported equation [18]: *predicted FVC volume (L) = 0.148 × height (in) – 0.025 × age (yr) – 4.241; predicted FEV₁ volume (L) = 0.092 × height (in) – 0.032 × age (yr) – 1.26.* The predicted percentages (%) of FVC (%FVC) and FEV_1 ($\%FEV_1$) were calculated as: *%predicted = measured volume (L) / predicted volume (L) × 100.* Test of pulmonary function was performed in the sitting position via closed circuit method, measuring inhaled and exhaled air at the same test cycle. Tests were carried out until gaining three adequate data. The criteria levels of %FVC predicted, $\%FEV_1$ predicted, and $\%FEV_1/FVC$ ratio were 80%, 80%, and 70%, respectively.

Chest radiography

Chest radiographs were obtained and scored according to the classification rules of the International Labor Office (ILO 2002) [19] by an experienced panel of physicians in the pneumoconiosis review committee of Korea Worker's Compensation & Welfare Service.

Table 1. General characteristics and 1-year progression of pneumoconiosis in retired coal miners with and without CWP

	Controls* (n = 22)	Subjects with CWP		p-values
		NP (n = 79)	P (n = 9)[§]	
Age, yrs	60.1/60.0 (34.1)	66.4/66.0 (62.5)	60.1/57.0 (42.3)	0.001[†]
BMI, kg/m²	22.3/22.0 (53.6)	22.4/22.2 (55.1)	23.0/22.7 (63.5)	0.720[†]
Exposure period, yrs	19.0/19.0 (56.0)	19.4/20.0 (57.0)	15.2/16.0 (41.4)	0.381[†]
%FVC predicted	90.2/92.1 (53.2)	93.1/91.6 (57.3)	88.9/87.8 (45.2)	0.519[†]
%FEV₁ predicted	83.6/86.5 (53.9)	87.1/89.1 (57.0)	83.5/82.3 (46.2)	0.605[†]
%FEV₁/FVC ratio	65.8/67.8 (58.6)	65.3/67.1 (54.1)	67.7/68.5 (60.5)	0.742[†]
Smoking, N (%)				
Never	5 (22.7)	13 (16.5)	1 (11.1)	0.091[‡]
Past	3 (13.6)	35 (44.3)	5 (55.6)	
Current	14 (63.6)	31 (39.2)	3 (33.3)	

*ILO classification 0/0 or 0/1, CWP: coal workers' pneumoconiosis, NP: without progression, P: with progression.
[†]Calculated by Kruskal-Wallis H test, arithmetic mean/median (mean rank).
[‡]Calculated by χ²- test, number of case (%).
[§]One-year progression of CWP was evaluated by paired comparison of chest-radiographs: made in 2007 and 2008. Category I (4 subjects): 1/0 (1) → 1/1, 1/1 (2) → 1/2, 1/1 (1) → 2/1, Category II (3 subjects): 2/1 (2) → 2/3, 2/2 (1) → 4A, Large opacity (2 subjects): 4A (2) → 4B.

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Statistical analyses

Levels of serum IL-8 and TNF-α were log-normally distributed. Therefore, cytokine data was log-transformed for parametric statistical tests, and the parametric unpaired student's t-test and ANOVA test were used to determine the magnitudes of between-group differences. If the sample sizes between control and test groups were also different or numbers of case each group were incompatible for parametric statistical tests, the nonparametric Mann-Whitney U test and Kruskal-Wallis one-way analysis based on rank sums were used to determine differences among the study groups. A multiple logistic regression model was constructed to compare controls with subjects with CWP, adjusted for age, BMI, exposure period, smoking status, and pulmonary function. Analysis by receiver operating characteristic (ROC) curve assessed the biomarker potential of each cytokine for the discrimination of the presence and progression of CWP. The best statistical levels of "cutoff" were calculated by minimizing the distance between the point with specificity = 1 and sensitivity = 1 and the points on the ROC curve. In the ROC analysis, an area under the curve (AUC) of 1.0 indicates perfect discrimination, whereas an area of 0.5 indicates that the test discriminates no better than chance. Values of $p < 0.05$ were considered statistically significant. All statistical evaluations were done with SPSS 14.0 (SPSS, Chicago, IL, USA).

Results

Study subjects

General characteristics of study subjects are shown in Table 1. In this study, 22 subjects were classified as controls and 88 were classified as CWP patients. Of the CWP patients, 79 and nine subjects were classified as non-progressive and progressive CWP, respectively, and 64 and 24 subjects were small and large opacity, respectively. The mean age was significantly higher in CWP without progression group compared with the other

Table 2. Concentrations of serum cytokines according to general characteristics

Characteristics		N	IL-8 (pg/ml)	p-values	TNF-α (pg/ml)	p-values
Age (yrs)*	-59	30	13.97 (1.66)	0.622	3.72 (1.28)	0.613
	60-69	49	15.84 (1.80)		3.85 (1.29)	
	70-	31	16.57 (2.75)		3.99 (1.40)	
BMI (kg/m²)†	< 25	86	15.50 (2.14)	0.998	3.82 (1.33)	0.609
	25 ≤	24	15.50 (1.64)		3.95 (1.29)	
Exposure period (yrs)‡	-9	12	14.75 (47.9)	0.146	3.62 (56.3)	0.647
	10-19	44	13.57 (48.9)		3.84 (56.8)	
	20-29	43	14.84 (61.8)		3.84 (56.9)	
	30-	11	16.76 (65.7)		3.67 (43.8)	
Smoking‡	Never	19	13.92 (51.0)	0.234	3.78 (53.2)	0.628
	Past	43	17.02 (62.0)		3.84 (59.2)	
	Current	48	13.93 (51.5)		3.73 (53.2)	
%FVC predicted†	80 ≤	90	15.53 (2.09)	0.949	3.80 (1.31)	0.328
	< 80	20	15.36 (1.79)		4.07 (1.33)	
%FEV₁ predicted†	80 ≤	76	14.24 (1.71)	0.056	3.70 (1.28)	0.022
	< 80	33	18.89 (2.69)		4.22 (1.38)	
%FEV₁/FVC ratio†	70 ≤	40	13.61 (1.83)	0.146	3.93 (1.28)	0.550
	< 70	70	16.70 (2.14)		3.80 (1.34)	

*Calculated by ANOVA test, geometric mean (geometric standard deviation).
†Calculated by t-test, geometric mean (geometric standard deviation).
‡Calculated by Kruskal-Wallis *H* test, median (mean rank).

two groups. Other general characteristics including averages of BMI, exposure period, and pulmonary function (%FVC predicted, %FEV$_1$ predicted, %FEV$_1$/FVC ratio), and smoking status did not show statistical differences among the study groups.

Concentrations of serum IL-8 and TNF-α

The mean serum TNF-α level was significantly higher (p = 0.022) in the subjects with low %FEV$_1$ predicted (< 80%) (4.22 pg/ml) *vs.* with normal %FEV$_1$ predicted (3.70 pg/ml) (Table 2). Although there was no statistical significance, the mean serum IL-8 level tended to increase with increasing age, and the mean serum IL-8 level in the subjects with low %FEV$_1$ predicted

(< 80%) tended to increase compared with normal %FEV$_1$ predicted. No differences were observed in the serum levels of IL-8 and TNF-α according to BMI, exposure period, smoking status, %FVC predicted, and %FEV$_1$/FVC ratio.

Serum cytokine levels according to CWP are shown in Table 3. The mean serum IL-8 level was significantly higher in subjects with CWP (16.75 pg/ml) *vs.* controls (11.38 pg/ml) in crude analysis (p = 0.022), and was both significantly and independently higher in adjusted logistic regression model (p = 0.007 for all comparisons). However, no differences were evident in the mean serum TNF-α level in subjects with CWP compared with controls (p = 0.379 for all comparisons).

In subjects with CWP, the median serum TNF-α level was

Table 3. Mean concentration of serum cytokines according to CWP

Cytokines	Controls (n = 22)	CWP (n = 88)	p-values	
			Univariate*	Multivariate[†]
IL-8, pg/ml	11.38 (1.55)	16.75 (2.11)	0.022	0.007
TNF-α, pg/ml	3.60 (1.32)	3.92 (1.32)	0.196	0.809

ILO classification 0/0 or 0/1, CWP: coal workers' pneumoconiosis.
*Calculated by student's t-test (healthy RCM vs. RCM with CWP), geometric mean (geometric standard deviation).
[†]Calculated from a multiple logistic regression model with serum level of each cytokines, adjusted for age, BMI, exposure period, smoking status, and pulmonary function (%FVC predicted, %FEV$_1$ predicted, and %FEV$_1$/FVC ratio).

Table 4. Median levels of serum cytokines according to progression of CWP

Cytokines	NP (n = 79)	P (n = 9)	p-values	
			Univariate*	Multivariate[†]
IL-8, pg/ml	15.43 (43.9)	16.29 (49.7)	0.522	0.379
TNF-α, pg/ml	3.84 (42.6)	4.60 (61.6)	0.040	0.027

ILO classification 0/0 or 0/1, CWP: coal workers' pneumoconiosis.
*Calculated by Mann-Withney *U* test, Median (Mean rank).
[†]Calculated from a multiple logistic regression model with serum level of each cytokines, adjusted for age, BMI, exposure period, smoking status, and pulmonary function (%FVC predicted, %FEV$_1$ predicted, and %FEV$_1$/FVC ratio).

Table 5. Area under the ROC curve for serum cytokines

Discrimination	Cytokines	AUROC	SE	95% CI	p-values	Cut off (pg/ml)	Sensitivity (%)	Specificity (%)
Presence of CWP*	IL-8	0.700	0.060	0.582-0.817	0.004	11.63	69.3	63.6
	TNF-α	0.592	0.069	0.458-0.727	0.181	-		
Progression of CWP[†]	IL-8	0.567	0.098	0.375-0.759	0.512	-		
	TNF-α	0.716	0.080	0.559-0.873	0.035	4.52	66.7	78.5

AURO: area under the ROC curve, SE: standard error, CWP: coal workers' pneumoconiosis, 95% CI: 95% confidence interval.
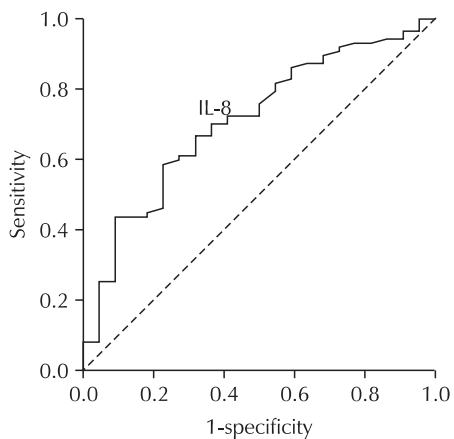*In all subjects (n = 110).
[†]In subjects with CWP (n = 88).

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Fig. 2. Receiver operating characteristics (ROC) curve of serum IL-8 for the diagnostic discrimination of CWP in all subjects (n = 110). The area under the ROC curve for serum IL-8 level was 0.70 (95% confidence interval, 0.58-0.82). The serum IL-8 level at 11.63 pg/ml was determined as the most optimal cutoff value with resulting sensitivity and specificity of 69.3% and 63.6% for the presence of CWP.
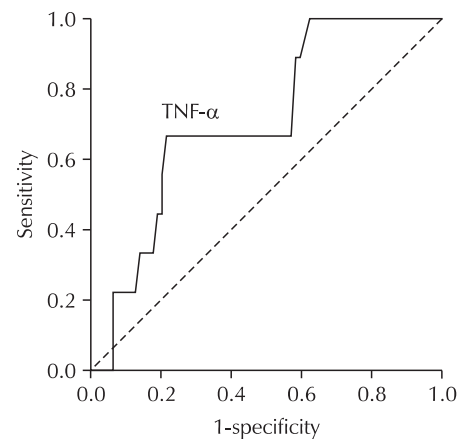
Fig. 3. Receiver operating characteristics (ROC) curve of serum TNF-α for the discrimination of progression of peumoconiosis in the subjects with CWP (n = 88). The area under the ROC curve for serum TNF-α level was 0.72 (95% confidence interval, 0.56-0.87). The serum TNF-α level at 4.52 pg/ml was determined as the most optimal cutoff value with resulting sensitivity and specificity of 66.7% and 78.5% for the progression of pneumoconiosis.

Table 6. Correlation coefficient between concentration of serum cytokines and independent variables (n = 110)

Variables	Age	BMI	Exposure period	X-ray profusion	%FVC	%FEV$_1$	%FEV$_1$/FVC	IL-8
BMI (kg/m^2)*	−0.033							
Exposure period*	0.040	0.085						
X-ray profusion[†,‡]	0.256[§]	0.060	−0.011					
%FVC*	−0.038	0.107	0.088	0.004				
%FEV$_1$*	−0.016	0.196[‖]	−0.030	−0.036	0.794[§]			
%FEV$_1$/FVC*	−0.249[‖]	0.191[‖]	−0.118	−0.097	0.197[‖]	0.722[§]		
IL-8*	0.084	−0.142	0.168	0.219[‖]	−0.065	−0.155	−0.180	
TNF-α*	0.082	−0.010	−0.177	0.169	−0.211[‖]	−0.149	−0.018	0.216[‖]

Cytokines were log transferred data.
*Pearson's product moment correlation coefficient (r), [§]p < 0.01, [‖]p < 0.05.
[†]Spearman's rank correlation coefficient (rho), [§]p < 0.01, [‖]p < 0.05.
[‡]Severity (ILO stage)of pneumoconiosis with small opacity (n = 86).

significantly higher in subjects with progressive CWP (4.60 pg/ml) than in those without progressive CWP (median, 3.84 pg/ml) in crude analysis (p = 0.040), was and both significantly and independently higher in adjusted logistic regression model (p = 0.027 for all comparisons). However, no difference was evident in the median serum IL-8 level in subjects with and without progressive CWP (p = 0.809 for all comparisons) (Table 4).

ROC analysis of serum IL-8 and TNF-α

The AUC of the ROC curve for serum IL-8 level was 0.70 (95% confidence interval, 0.58-0.82). The serum IL-8 level of 11.63 pg/ml was determined to be the optimal cutoff value with resulting sensitivity and specificity of 69.3% and 63.6%, respectively, for the presence of CWP. The AUC of the ROC curve for serum TNF-α level was 0.72 (95% confidence interval, 0.56-0.87). The serum TNF-α level of 4.52 pg/ml

Table 7. Stepwise multiple regression analysis of serum cytokines against associated variables

Variables		B	SE	β	p-values
Dependants	Independents				
Log (IL-8)	Constant	1.056	0.048		0.000
	Presence of CWP	0.118	0.056	0.223	0.039
	R^2	0.223			
	Adjusted R^2	0.050			
	F	4.411 (p = 0.039)			
Log (TNF-α)	Constant	0.568	0.012		0.000
	Progression of CWP	0.092	0.043	0.229	0.034
	R^2	0.229			
	Adjusted R^2	0.059			
	F	4.644 (p = 0.034)			

Subjects: controls and CWP patients with small opacity (n = 86).
B: regression coefficients, SE: standard error, β: standardized B, unit: pg/ml.
Independent variables: age, BMI, exposure period, smoking status (yes/no), presence of CWP (yes/no), progression of CWP (yes/no), %FVC predicted, %FEV$_1$ predicted, %FEV$_1$/FVC ratio, and serum cytokines (IL-8, and TNF-α).

was determined as the optimal cutoff value with resulting sensitivity and specificity of 66.7% and 78.5%, respectively, for the progression of CWP (Table 5, Figs 2, 3).

Relationships between serum cytokines levels and associated variables

Serum IL-8 levels correlated with pneumoconiosis classifications in the CWP subjects with small opacity (rho = 0.219, p < 0.05) and serum TNF-α levels (r = 0.216, p < 0.05) (Table 6). Serum TNF-α levels correlated with %FVC predicted (r = 0.211, p < 0.05). Stepwise multiple regression analysis (Table 7) revealed that the serum IL-8 levels were positively related with the presence of CWP (adjusted R^2 = 0.050, β = 0.223, p = 0.039), and a positive relation of serum TNF-α levels with the progression of CWP (adjusted R^2 = 0.059, β = 0.229, p = 0.034).

Discussion

CWP results from exposure to coal mine dust and is characterized by a progressive fibrotic reaction in the lung that can cause functional damage and irreversible change [1]. Fibrosis is a disorder characterized by a qualitative and quantitative alteration of the deposition of extracellular matrix with accumulation of mesenchymal cells in replacement of normal tissue. The sequence of events leading to fibrosis of an organ involves the subsequent processes of injury with inflammation

and disruption of the normal tissue architecture, followed by tissue repair with accumulation of mesenchymal cells in this area [20]. Reactive oxygen species and cytokines may play important roles in mineral dust exposure and related lung disorders. However, the exact consequences of the mechanisms that occur in the lungs of subjects chronically exposed to coal dust are still unclear [1]. Castranova and Vallyathan [21] proposed that the development and progression of CWP occurs with four basic mechanisms: a) direct cytotoxicity of coal dust or silica; b) activation of oxidant production by pulmonary phagocytes; c) activation of mediator release from alveolar macrophages and epithelial cells, which leads to recruitment of polymorphonuclear leukocytes and macrophages, resulting in the production of proinflammatory cytokines and reactive species and in further lung injury and scarring; and d) secretion of growth factors from alveolar macrophages and epithelial cells, stimulating fibroblast proliferation and eventual scarring. The risk of CWP depends on the concentration and duration of exposure to coal dust that usually contains relatively small amounts of free crystalline silica (quartz). McCunney et al. [22] proposed that the active agent within coal is iron rather than quartz. Therefore, by identifying components of coal before mining activities, the risk of developing CWP might be reduced.

Alcohol alters cytokine levels in the blood and in a variety of tissues [23]. Acute ethanol intoxication may inhi-

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bit the production of IL-8 and TNF-α [24], and the abnormalities of production of inflammatory cytokines in chronic alcoholic patients observed depending on both the status of ethanol intake and the existence of alcoholic liver disease [25]. Although we presently lacked information on the alcohol consumption of the study subjects, the demonstrated physiologic and pathologic coordination of serum cytokines [26-28] prompted the exclusion of subjects who had exceed the criteria level related to the function of liver in serum, including aspartate aminotransferase, alanine aminotransferase, and gamma-glutamyl transpeptidase, because measurement of liver function is a standard diagnostic tool for the identification of chronic alcohol exposure [29]. We also excluded the subjects who had exceed the criteria level related to the function of kidney in serum, including blood urea nitrogen and creatinine [30,31].

Identification of biomarkers that are accurate and reliable in the prediction and early detection of CWP are imperative for the implementation of timely intervention strategies [3]. The present study was performed using one-year (2007-2008) prospective data and cross-sectional findings. The alveolar macrophage may be the first cell encountered by the invading organism and become activated. The activated alveolar macrophage will produce not only IL-8, but also early response cytokines such as TNF and IL-1. With the developing inflammatory response and increased permeability of the alveolar capillary membrane, TNF and IL-1 could stimulate other cells of membrane (pulmonary epithelial cells, interstitial fibroblasts, smooth-muscle cells, and endothelial cells) to establish a significant IL-8 chemotactic gradient, culminating in the recruitment of neutrophils into the lung parenchyma [32]. Useful biomarkers for crystalline silica- and CWP-pneumoconiosis that have been identified include IL-8 and TNF-α released from monocytes, Clara cell protein in serum, reactive oxygen species, and various antioxidants [3].

IL-8 is a member of a structurally similar family of cytokines designated chemokines, which display chemotactic activity for neutrophils. IL-8 has been implicated in a variety of inflammatory diseases. Various cells express IL-8 mRNA and rapidly produce IL-8 protein, including monocytes, T lymphocytes, neutrophils, fibroblasts, endothelial cells, and epithelial cells. IL-8 affects the adhesion of neutrophils to the endothelium and induces the transendothelial migration of neutrophils [13]. IL-8 is produced in response to proinflammatory stimuli. The accumulation of inflammatory leukocytes in the lung is a hallmark of either acute or chronic pulmonary inflammation [32]. Strieter et al. [12] reported the central role of the alveolar macrophage in the elicitation of polymor-

phonuclear cells into the lung via the production of IL-8. Although TNF has no direct neutrophil chemoattractant activity, which is potent inducer of IL-8 production in a variety of cell types, including alveolar macrophages, epithelial cells, lung fibroblasts, and endothelial cells [33,34]. Presently, serum IL-8 levels weakly correlated with serum TNF-α levels (p < 0.05). IL-8 is important in crystalline silica-induced lung inflammation [11] and its level is increased in the supernatant of spontaneous or coal-stimulated monocytes from peripheral blood and in sera of CWP patients [14]. Presently, the mean serum IL-8 level was significantly higher in CWP patients compared to controls (p = 0.007 for all comparisons). Serum IL-8 levels correlated with pneumoconiosis classifications in the subjects with small opacity (p < 0.05) and were positively related with the presence of CWP (p = 0.039). IL-8 is a chemoattractant cytokine having distinct target specificity for the neutrophil. However, it is also active on primed eosinophils and is involved in neutrophilic inflammation in asthma and chronic bronchitis [35], and IL-8 levels may be relevant to asthma pathogenesis [36]. Keatings et al. [37] suggested that IL-8, but not TNF-α, is significantly higher in patients with chronic obstructive pulmonary disease (COPD) than asthmatics, and that the TNF-α and IL-8 cytokines may be involved in COPD-related inflammation. In this study, serum IL-8 levels, but not TNF-α, tended to increase with severities of COPD (data not shown).

TNF-α is a proinflammatory cytokine that is important in the early onset of inflammation, development, and progression of several diseases including pulmonary fibrosis. TNF-α can be released by a number of cell types including activated macrophages, monocytes, and polymorphonuclear cells [1]. They are initiators of cytokine networks and lead to neutrophil recruitment and chemotaxis [38]. TNF-α has various roles that include synergistic effects in inflammatory and immune responses [3]. TNF-α would be responsible for the initiation and perpetuation of the inflammatory reaction observed in the lung of patients with progressive massive fibrosis. TNF-α, which can directly induce fibroblast proliferation, can also trigger the production of mediators [39]. The serum level of TNF-α level tends to increase in CWP [40]. TNF-α has been reported as a predicted biomarker for progressive pneumoconiosis, with a level that correlates with severity of pneumoconiosis [11], and as a useful index for coal dust exposure and a biomarker for pneumoconiosis with progressive fibrosis in the lung [3]. In the present study, the median serum TNF-α level was significantly higher in subjects with progression of CWP compared with subjects without progression (p = 0.027 for all comparisons). The serum TNF-α levels were positively related with the progression of CWP (p = 0.034).

Applying ROC curve analyses, AUC for IL-8 (0.70) values for the identification of CWP and TNF-α (0.72) for the prediction of progression of CWP indicated the reasonable potential of IL-8 and TNF-α as biomarkers. The cutoff values of serum IL-8 and TNF-α were 11.63 pg/ml (sensitivity 69%; specificity 64%) and 4.52 pg/ml (sensitivity 67%; specificity 79%) respectively. These results suggest that levels of serum IL-8 and TNF-α might serve as biomarkers for the presence of CWP and for the progression of CWP, respectively.

Although pneumoconiosis is the most prevalent lung disease that produces decreased pulmonary function and emphysema [1], the present difference between measured cytokines and PFT was not compelling, unless TNF-α showed a significant difference according to criteria level (80%) of %FEV$_1$ predicted. The best explanation for these observations is that decreased PFT is the result of inflammation or fibrosis in the lung but cytokines influence the current inflammatory response.

The study has several limitations. One is that the follow-up period of 1 year was insufficient, especially in the absence of control data on subjects not exposed to mine dust. Another limitation concerns the lack of data of co-affective factors such as serum leptin related with TNF-α [41,42] and neurotrophic factor related with IL-8 [43]. Serum cytokines can be measured by various techniques that include enzyme-linked immunosorbent assay (ELISA), cytokine flow cytometry, and biochip array [44]. The mean serum TNF-α level determined using a biochip array was higher in controls and subjects with CWP compared with those reported by Zhai et al. [45] (1.7 and 1.4 pg/ml in affected subjects and controls, respectively), in coal miners with pneumoconiosis using ELISA. Optimal cutoff values may, therefore, differ depending on the method of analysis. However, presently, the serum levels of IL-8 and TNF-α significantly differed according to the presence or the progression of CWP, and the data in ROC analysis for the presence or the progression of CWP indicated the potential of IL-8 and TNF-α as biomarkers.

In conclusion, high levels of serum IL-8 are associated with the presence of CWP and those of serum TNF-α are associated with the progression of CWP. Further longitudinal follow-up studies are needed to investigate the potential of serum cytokines as useful biomarker of CWP, and future studies will be required to ascertain the cytokine profile in pneumoconiosis patients using lung specific specimens such as bronchoalveolar lavage fluid, exhaled breath condensate, or lung tissue.

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