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# Blood Levels of IL-1β, IL-6, IL-8, TNF-α, and MCP-1 in Pneumoconiosis Patients Exposed to Inorganic Dusts

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Inhaled inorganic dusts such as coal can cause inflammation and fibrosis in the lung called pneumoconiosis. Chronic inflammatory process in the lung is associated with various cytokines and reactive oxygen species (ROS) formation. Expression of some cytokines mediates inflammation and leads to tissue damage or fibrosis. The aim of the present study was to compare the levels of blood cytokines interleukin (IL)-1β, IL-6, IL-8, tumor necrosis factor (TNF)-α and monocyte chemoattractant protein (MCP)-1 among 124 subjects (control 38 and pneumoconiosis patient 86) with category of chest x-ray according to International Labor Organization (ILO) classification. The levels of serum IL-8 ($p = 0.003$), TNF-α ($p = 0.026$), and MCP-1 ($p = 0.010$) of pneumoconiosis patients were higher than those of subjects with the control. The level of serum IL-8 in the severe group with the small opacity (ILO category II or III) was higher than that of the control ($p = 0.035$). There was significant correlation between the profusion of radiological findings with small opacity and serum levels of IL-1β (*rho* = 0.218, $p < 0.05$), IL-8 (*rho* = 0.224, $p < 0.05$), TNF-α (*rho* = 0.306, $p < 0.01$), and MCP-1 (*rho* = 0.213, $p < 0.01$). The serum levels of IL-6 and IL-8, however, did not show significant difference between pneumoconiosis patients and the control. There was no significant correlation between serum levels of measured cytokines and other associated variables such as lung function, age, BMI, and exposure period of dusts. Future studies will be required to investigate the cytokine profile that is present in pneumoconiosis patient using lung specific specimens such as bronchoalveolar lavage fluid (BALF), exhaled breath condensate, and lung tissue.

***Key words***: Cytokine, Lung inflammation, Pneumoconiosis

## INTRODUCTION

Among occupational lung diseases, most prevalent diseases are induced by inhalation of dusts such as asbestos, crystalline silica and coal. Inhalation of theses dusts may cause a variety of lung diseases such as progressive massive fibrosis (PMF), chronic alveolitis, emphysema, and coal workers pneumoconiosis (CWP). Fibrosis of tissue resulting from theses dusts may invoke functional damage and irreversible change (Schins and Borm, 1999). Notably, crystalline silica has been classified as class I carcinogen by the International Agency for Research on Cancer (IARC, 1997).

Pneumoconiosis is a lung disease caused by inhaling 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 fibrosis in the lung, which is usually irreversibly progressive. Once silica threshold has been exceed, 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 carried out before irreversible radiological changes in the lung (Gulumian *et al.*, 2006; Porter *et al.*, 2004). Many researchers have studied the role of mediators such as a various cytokines and reactive oxygen species (ROS) in pulmonary inflammation resulting from mineral dusts (Schins and Borm, 1999).

Cytokines have an effect on various biological events such as inflammation, metabolic mechanism, growth

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and proliferation of the cell, morphogenesis, fibrosis, and homeostasis (Elias and Zitnik, 1992). Major sources of cytokines in the lung are epithelial cells, endothelial cells, fibroblasts and inflammatory cells. In studies for relationship between pulmonary inflammation and dusts, cytokines have been demonstrated for mediator of a various toxicological and pathological effects. The cytokines associated with coal dust exposure were IL-1, IL-4, IL-6, IL-8, IL-10, IL-11, IL-12, IL-13, TNF-$\alpha$, MCP-1, transforming growth factor-$\beta$, insulin-like growth factor-$\beta$, and platelet derived growth factor (Ates *et al.*, 2008; Griwatz *et al.*, 1994; Griwatz and Seemayer, 1994; Prince *et al.*, 2008; Razzaque and Taguchi, 2003; Vanhee *et al.*, 1995; Weber *et al.*, 1996; Ulkor *et al.*, 2008). Although there were a few reports of the relationship between blood cytokines and radiological findings in Korea, these reports not included the pulmonary function test (PFT) and were also controversy about the validity of the radiological findings.

The objective of this study is to investigate the relationship between blood cytokines (IL-1$\beta$, IL-6, IL-8, TNF-$\alpha$, and MCP-1) as inflammation mediator and pneumoconiosis findings obtained from radiological findings identified by the pneumoconiosis review committee and PFT.

## MATERIALS AND METHODS

***Subjects.*** The study population contained 124 retired male workers exposed to inorganic dust, who had lower criteria level related to liver and kidney function such as aspartate aminotransferase, alanine aminotransferase, gamma-glutamyl transpeptidase, blood urea nitrogen, and creatinine. We carried out collecting of blood and urine, chest x-ray, and PFT under the informed consent from all of subjects. Personal information on age, body weight, height, various personal history (job, smoking status and disease) was obtained by a structured questionnaire. The study was approved from the Research Ethics Committee of our research center.

***Analysis of blood cytokines.*** Serum was centrifuged at 3,000 rpm for 10 min. The samples were stored at -80°C until assay. Analysis of blood cytokines was measured by biochip array (EV 3513, Randox Laboratories Ltd., U.K.) using sandwich & competitive chemiluminescence immunoassay, as previously described (Fitzgerald *et al.*, 2005; Molloy *et al.*, 2005). Detection limits of IL-1$\beta$, IL-6, IL-8, TNF-$\alpha$, and MCP-1 were 0.6, 0.4, 1.5, 2.6, and 2.5 pg/ml, respectively.

***Clinical indices of liver and kidney.*** Because inflammation mediator in the blood is influenced by inflammation of whole body, AST, ALT, and $\gamma$-GT were measured as biochemical indicator for liver, and BUN and creatinine were measured as that for kidney using automated biochemical analyzer (Hitachi 7080, Hitachi, Japan) (Brunzel, 2003; Ingram, 2003).

***PFT.*** PFT was performed in accordance with recommended guideline of ATS/ERS Task Force (Brusasco *et al.*, 2005) using spirometer (Vmax22, SensorMedics, USA). We measured 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 ($FEV_1$), which is the volume delivered in the first second of an FVC maneuver, and $FEV_1$/FVC ratio, and calculated predicted volume by regression equation of Morris *et al.* (1971).

predicted volume (L) = 0.0583 × height (in) - 0.025 × age (yr) - 4.241

The predicted percentages (%) of FVC and $FEV_1$ calculated by following way,

% 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 3 adequate data.

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

***Statistical analysis.*** The data was analyzed using SPSS 14.0 (SPSS, Chicago, IL, USA). General characteristics and PFT data showed normal distribution, whereas the measured cytokines showed log-normal distribution (Kolmogorov-Smirnov test); therefore the data of cytokines were log-transformed for all of the statistical tests, and the results were expressed as the

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

| ILO category | N | % | Profusion (N) |
|---|---|---|---|
| Control | 38 | 30.6 | 0/0 (32); 0/1 (6) |
| Small opacity | 67 | 54.1 | |
| I | 40 | 32.3 | 1/0 (11); 1/1 (22); 1/2 (7) |
| II, III | 27 | 21.8 | 2/1 (11); 2/2 (14); 2/3 (1); 3/2 (1) |
| Large opacity | 19 | 15.3 | A (17); B (2) |

geometric mean (GM) and geometric standard deviation (GSD). We analyzed log-transformed data using One-way analysis of variance (ANOVA) followed by Turkey's HSD comparison or t-test. Pearson's product moment correlation coefficient (*r*) was used to assess the correlations between measured cytokines and the considered groups of subjects except for the x-ray profusions expressed Spearman's rank correlation coefficient (*rho*). A *p*-value of $< 0.05$ (two-tailed) was considered significant for all of the tests.

**Table 2.** General characteristics of the study subjects

| | Control (n=38) | Pneumoconiosis | | *p*-values |
|---|---|---|---|---|
| | | Small opacity (n = 67) | Large opacity (n = 19) | |
| Age (yrs) | 61.2 ± 7.2 | 63.6 ± 7.7 | 66.0 ± 7.5 | 0.065[1] |
| BMI (kg/m$^2$) | 22.4 ± 3.0 | 22.4 ± 2.7 | 21.3 ± 2.4 | 0.300[1] |
| Exposure period (yrs) | 17.8 ± 7.7 | 19.4 ± 7.9 | 17.1 ± 6.9 | 0.408[1] |
| FVC, % predicted | 93.0 ± 12.7 | 92.8 ± 13.8 | 92.2 ± 11.5 | 0.974[1] |
| $FEV_1$, % predicted | 88.4 ± 18.2 | 87.7 ± 20.1 | 84.3 ± 15.8 | 0.727[1] |
| $FEV_1$/FVC ratio (%) | 72.0 ± 1.8 | 71.5 ± 1.9 | 70.8 ± 1.7 | 0.068[1] |
| Smoking, N (%) | | | | |
| Never | 8 (47.1) | 7 (41.2) | 2 (11.8) | 0.115[2] |
| Past | 8 (17.4) | 31 (67.4) | 7 (15.2) | |
| Current | 22 (36.1) | 29 (47.5) | 10 (16.4) | |

Arithmetic mean ± standard deviation
[1]Calculated by ANOVA test
[2]Calculated by $\chi^2$-test

**Table 3.** Concentrations of blood cytokines according to general characteristics

| Characteristics | | N | IL-1β | IL-6 | IL-8 | TNF-α | MCP-1 |
|---|---|---|---|---|---|---|---|
| Age (yrs)[1] | ~49 | 7 | 1.51 (2.54) | 1.15 (2.50) | 17.62 (1.77) | 4.68 (1.45) | 185.4 (1.4) |
| | 50~59 | 30 | 1.30 (2.04) | 1.23 (3.71) | 13.43 (1.55) | 3.74 (1.30) | 188.5 (1.6) |
| | 60~69 | 61 | 1.22 (3.47) | 1.31 (2.42) | 14.82 (1.74) | 3.91 (1.33) | 191.2 (1.5) |
| | 70~ | 26 | 0.86 (2.18) | 1.32 (2.07) | 15.40 (1.67) | 3.84 (1.39) | 177.5 (1.3) |
| | | | $p = 0.379$ | $p = 0.980$ | $p = 0.579$ | $p = 0.333$ | $p = 0.874$ |
| BMI (kg/m$^2$)[2] | 25 ≤ | 22 | 1.11 (3.89) | 1.19 (2.23) | 12.45 (1.71) | 3.77 (1.31) | 159.8 (1.3) |
| | < 25 | 102 | 1.18 (2.61) | 1.30 (2.72) | 15.27 (1.67) | 3.92 (1.35) | 193.8 (1.5) |
| | | | $p = 0.794$ | $p = 0.680$ | $p = 0.095$ | $p = 0.560$ | $p = 0.031$ |
| Exposure period (yrs)[1] | ~9 | 16 | 1.60 (4.96) | 1.15 (2.16) | 13.81 (1.54) | 4.12 (1.31) | 175.2 (1.4) |
| | 10~19 | 51 | 1.22 (2.77) | 1.41 (3.44) | 13.38 (1.51) | 3.87 (1.40) | 200.6 (1.5) |
| | 20~29 | 42 | 0.97 (2.28) | 1.31 (2.14) | 17.01 (1.94) | 3.89 (1.31) | 187.3 (1.4) |
| | 30~ | 15 | 1.19 (2.34) | 0.99 (1.78) | 14.61 (1.50) | 3.73 (1.26) | 159.1 (1.6) |
| | | | $p = 0.417$ | $p = 0.626$ | $p = 0.156$ | $p = 0.817$ | $p = 0.182$ |
| Smoking[1] | Never | 17 | 0.78 (2.03) | 0.96 (2.03) | 13.31 (1.53) | 3.77 (1.27) | 167.4 (1.3) |
| | Past | 46 | 1.12 (3.02) | 1.26 (2.08) | 15.24 (1.69) | 4.19 (1.32) | 183.8 (1.6) |
| | Current | 61 | 1.35 (2.82) | 1.41 (3.19) | 14.77 (1.72) | 3.71 (1.36) | 196.0 (1.4) |
| | | | $p = 0.143$ | $p = 0.339$ | $p = 0.659$ | $p = 0.094$ | $p = 0.298$ |
| %FVC predicted[2] | 80 ≤ | 103 | 1.23 (2.93) | 1.29 (2.82) | 14.46 (1.65) | 3.95 (1.34) | 185.5 (1.5) |
| | < 80 | 21 | 0.91 (2.16) | 1.25 (1.65) | 16.11 (1.83) | 3.61 (1.34) | 196.6 (1.4) |
| | | | $p = 0.223$ | $p = 0.882$ | $p = 0.388$ | $p = 0.198$ | $p = 0.527$ |
| %$FEV_1$ predicted[2] | 80 ≤ | 87 | 1.21 (3.04) | 1.22 (2.87) | 14.16 (1.63) | 3.76 (1.29) | 181.2 (1.5) |
| | < 80 | 37 | 1.08 (2.29) | 1.45 (2.04) | 16.15 (1.78) | 4.23 (1.43) | 202.4 (1.5) |
| | | | $p = 0.566$ | $p = 0.348$ | $p = 0.198$ | $p = 0.072$ | $p = 0.141$ |
| %$FEV_1$/FVC ratio[2] | 70 ≤ | 100 | 1.25 (2.88) | 1.24 (2.53) | 14.69 (1.69) | 3.95 (1.32) | 183.7 (1.4) |
| | < 70 | 24 | 0.88 (2.46) | 1.46 (3.10) | 14.90 (1.64) | 3.64 (1.41) | 202.8 (1.6) |
| | | | $p = 0.136$ | $p = 0.460$ | $p = 0.903$ | $p = 0.222$ | $p = 0.258$ |

Geometric mean (Geometric standard deviation), unit: pg/ml
[1]Calculated by ANOVA test
[2]Calculated by t-test

**Table 4.** Concentrations of IL-1β, IL-6, IL-8, TNF-α and MCP-1 in the blood of pneumoconiosis patients

| Cytokines | | N | GM[1] | GSD | Range | *p*-values of difference[2] | |
|---|---|---|---|---|---|---|---|
| | | | | | | 2 | 3 |
| IL-1β | 1. Control | 38 | 1.24 | 3.16 | ND[3]~201.33 | 1.000 | 1.000 |
| | 2. Small opacity | 67 | 1.14 | 2.77 | ND~68.57 | | 1.000 |
| | 3. Large opacity | 19 | 1.11 | 2.40 | ND~6.66 | | |
| | | | F = 0.105 ($p$ = 0.900) | | | | |
| IL-6 | 1. Control | 38 | 1.09 | 2.63 | 0.36~17.41 | 0.900 | 0.750 |
| | 2. Small opacity | 67 | 1.34 | 2.48 | 0.38~193.09 | | 1.000 |
| | 3. Large opacity | 19 | 1.50 | 3.18 | 0.55~74.33 | | |
| | | | F = 0.832 ($p$ = 0.438) | | | | |
| IL-8 | 1. Control | 38 | 11.97 | 1.78 | 3.92~66.33 | 0.042 | 0.004 |
| | 2. Small opacity | 67 | 15.42 | 1.56 | 7.66~48.31 | | 0.333 |
| | 3. Large opacity | 19 | 18.98 | 1.66 | 5.20~46.18 | | |
| | | | F = 6.009 ($p$ = 0.003) | | | | |
| TNF-α | 1. Control | 38 | 3.57 | 1.29 | 2.29~6.98 | 0.286 | 0.024 |
| | 2. Small opacity | 67 | 3.94 | 1.33 | 2.02~10.95 | | 0.332 |
| | 3. Large opacity | 19 | 4.44 | 1.42 | 2.08~8.39 | | |
| | | | F = 3.768 ($p$ = 0.026) | | | | |
| MCP-1 | 1. Control | 38 | 165.9 | 1.4 | 49.9~348 | 0.238 | 0.008 |
| | 2. Small opacity | 67 | 189.6 | 1.4 | 62.6~900 | | 0.162 |
| | 3. Large opacity | 19 | 228.7 | 1.6 | 118.7~900 | | |
| | | | F = 4.818 ($p$ = 0.010) | | | | |

[1]GM: geometric mean, GSD: geometric standard deviation, unit: pg/ml
[2]p-values: calculated by ANOVA (Turkey's HSD) test
[3]Not detection: lower than limit of detection (LOD of IL-1β: 0.6 pg/ml)

## RESULTS

***General characteristics of the study populations.*** Number of study subjects by ILO categories of pneumoconiosis (ILO, 2002) was "small opacity" in 67 (54.1%), type in 40 (29.4%) and type II in 28 (20.6%), and "large opacity" in 19 (15.3%) (Table 1).

The characteristics of the study populations are showed in Table 2. The mean of age in the control (n = 38), the small opacity (n = 67), and the large opacity (n = 19) were 61.2 ± 7.2, 63.6 ± 7.7, and 66.0 ± 7.5, respectively. Body mass index (BMI), exposure period, pulmonary function, and smoking status did not show statistical difference between the control and pneumoconiosis groups.

***Concentration of blood cytokines according to general characteristics.*** The mean concentration of MCP-1 in low BMI (< 25 kg/m$^2$) was higher than that in high BMI (≥ 25 kg/m$^2$) ($p$ = 0.031). There were no significant differences between levels of IL-1β, IL-6, IL-8, and TNF-α in blood and general characteristics such as age, exposure period, smoking status. Furthermore there were no significant differences between levels of measured cytokines in blood and criteria levels of %FVC, %FEV$_1$ and %FEV$_1$/FVC (Table 3).

***Concentration of blood cytokine according to ILO categories of pneumoconiosis.*** The mean concentrations of IL-8 ($p$ = 0.003), TNF-α ($p$ = 0.026), and MCP-1 ($p$ = 0.010) in pneumoconiosis groups (small or large opacity) were higher than those of the control (Table 4). IL-8 levels in the small opacity, large opacity and the control were 15.42 pg/ml, 18.98 pg/ml, and 11.97 pg/ml, respectively. IL-8 levels in the small opacity ($p$ = 0.042) and large opacity ($p$ = 0.004) were higher than those of the control.

The mean concentrations of TNF-α (4.44 pg/ml) ($p$ = 0.024) and MCP-1 (228.7 pg/ml) ($p$ = 0.008) in the large opacity were higher than those of the control (3.57 pg/ml and 165.9 pg/ml, respectively). Although there were no statistical significance, TNF-α and MCP-1 levels in the small opacity tended to increase comparing with the control. For the IL-1β and IL-6 levels, there were no statistical significance between the control and pneumoconiosis groups.

The level of serum IL-8 in the severe group with the small opacity (ILO category II or III) was higher than that of the control (16.41 pg/ml *vs* 11.97 pg/ml, $p$ = 0.035) (Table 5).

***Correlation between concentration of blood cytokine and associated variables.*** As shown in Table 6,

**Table 5.** Concentrations of blood cytokines according to x-ray profusion in pneumoconiosis patients with small opacity

| Cytokines | X-ray profusion† | N | GM[1] | GSD | Range | *p*-values of difference[2] | |
|---|---|---|---|---|---|---|---|
| | | | | | | 2 | 3 |
| IL-1β | 1. 0 | 38 | 1.24 | 3.16 | ND[3]~201.33 | 0.977 | 0.881 |
| | 2. I | 40 | 1.14 | 2.77 | ND~68.57 | | 0.953 |
| | 3. II, III | 27 | 1.09 | 2.60 | ND~116.66 | | |
| | | | F = 0.115 ($p$ = 0.892) | | | | |
| IL-6 | 1. 0 | 38 | 1.09 | 2.63 | 0.36~17.41 | 0.632 | 0.619 |
| | 2. I | 40 | 1.33 | 2.99 | 0.38~193.09 | | 0.993 |
| | 3. II, III | 27 | 1.36 | 1.73 | 0.57~3.68 | | |
| | | | F = 0.586 ($p$ = 0.558) | | | | |
| IL-8 | 1. 0 | 38 | 11.97 | 1.78 | 3.92~66.33 | 0.154 | 0.035 |
| | 2. I | 40 | 14.78 | 1.66 | 7.66~48.31 | | 0.674 |
| | 3. II, III | 27 | 16.41 | 1.41 | 9.08~32.26 | | |
| | | | F = 3.482 ($p$ = 0.034) | | | | |
| TNF-α | 1. 0 | 38 | 3.57 | 1.29 | 2.29~6.98 | 0.276 | 0.317 |
| | 2. I | 40 | 3.93 | 1.35 | 2.23~10.95 | | 0.998 |
| | 3. II, III | 27 | 3.95 | 1.30 | 2.02~7.26 | | |
| | | | F = 1.539 ($p$ = 0.219) | | | | |
| MCP-1 | 1. 0 | 38 | 165.9 | 1.4 | 49.9~348.0 | 0.322 | 0.174 |
| | 2. I | 40 | 186.2 | 1.5 | 86.7~900.0 | 0.868 | |
| | 3. II, III | 27 | 194.7 | 1.4 | 62.6~322.6 | | |
| | | | F = 1.864 ($p$ = 0.160) | | | | |

†ILO Categories (0: 0/0, 0/1; I: 1/0, 1/1, 1/2; II: 2/1, 2/2, 2/3, III: 3/2)
[1]GM: geometric mean, GSD: geometric standard deviation, unit: pg/ml
[2]*p*-values: calculated by ANOVA (Turkey's HSD) test
[3]Not detection: lower than limit of detection (LOD of IL-1β: 0.6 pg/ml)

**Table 6.** Correlation coefficient between blood levels of cytokines concentration and independent variables

| | N | IL-1β | IL-6 | IL-8 | TNF-α | MCP-1 |
|---|---|---|---|---|---|---|
| Age (yrs)[1] | 124 | 0.005 | -0.152 | 0.048 | 0.076 | 0.011 |
| Exposure period (yrs)[1] | 124 | -0.101 | -0.141 | -0.038 | 0.103 | -0.090 |
| BMI (kg/m$^2$)[1] | 124 | -0.090 | -0.111 | -0.065 | -0.109 | -0.105 |
| Smoking habit (pack yrs)[1] | 124 | 0.221 | 0.070 | -0.028 | 0.001 | 0.030 |
| FVC, % predicted[1] | 124 | -0.043 | 0.036 | -0.019 | -0.126 | -0.103 |
| $FEV_1$, % predicted[1] | 124 | -0.140 | 0.047 | -0.095 | -0.106 | -0.169 |
| $FEV_1$/FVC ratio %[1] | 124 | -0.004 | 0.159 | -0.088 | -0.061 | -0.069 |
| X-ray Profusion[2a] | 105 | 0.218* | -0.004 | 0.224* | 0.306** | 0.213* |

All cases were Log transformed data
* $p$<0.05, ** $p$<0.01,
[1]Pearson's product moment correlation coefficient ($r$)
[2]Spearman's rank correlation coefficient (*rho*)
[a]Severity of pneumoconiosis with small opacity

**Table 7.** Correlation matrix of blood cytokine concentration (N = 124)

| | IL-1β | IL-6 | IL-8 | TNF-α | MCP-1 |
|---|---|---|---|---|---|
| IL-1β | 1.000 | | | | |
| IL-6 | 0.201* | 1.000 | | | |
| IL-8 | -0.071 | 0.048 | 1.000 | | |
| TNF-α | 0.188* | 0.229* | 0.206* | 1.000 | |
| MCP-1 | -0.005 | 0.300** | 0.255** | 0.116 | 1.000 |

All cases were Log transformed data
Pearson's product moment correlation coefficient
(*$p$ < 0.05, **$p$ < 0.01)

there was significant correlation between blood cytokines, including TNF-α (*rho* = 0.306, $p$ < 0.01), IL-1β (*rho* = 0.218, $p$ < 0.05), IL-8 (*rho* = 0.224, $p$ < 0.05), and MCP-1 (*rho* = 0.213, $p$ < 0.01), and pneumoconiosis severities in small opacity.

Correlation matrix among all cytokine levels was showed in Table 7. TNF-α correlated with IL-6 ($r$ = 0.229, $p$ < 0.01) and IL-1β ($r$ = 0.188, $p$ < 0.05), and IL-8 also correlated with MCP-1 ($r$ = 0.255, $p$ < 0.01) and TNF-α ($r$ = 0.206, $p$ < 0.05). MCP-1 correlated with IL-6

($r = 0.300$, $p < 0.01$).

## DISCUSSION

Toxicity and interaction of crystalline silica and coal dust are based on activation of macrophages and lung inflammation, many researchers have concerned about crucial mediators for the pulmonary disorder resulting from these mineral dust (Schins and Borm, 1999).

Inhaled dust leads to generation of ROS resulting from activated phagocytes in the lung, and transitional metals, including iron, copper, and vanadium were positively correlated with generation of ROS (Becker *et al.*, 1996; Castranova *et al.*, 1997; Dalal *et al.*, 1991; Shoemaker *et al.*, 1995; Tourmann and Kaufmann, 1994; Vallyathan, 2004; Wallaert *et al.*, 1990).

Inhaled inorganic particles cause release of cytokines resulting in inflammation and injury to lung epithelial cell (Lasky *et al.*, 2005). Recruitment of inflammatory cells such as monocytes, macrophages, and neutrophils plays an important role in inflammatory process in the lung. Inflammation and its progression may depend upon dust concentration and it is proceeded after discontinuation of exposure (Donaldson *et al.*, 1990). Chronic injury involving loss of phagocytosis may continuously lead to inflammation with persistent oxidative stress (Perlman *et al.*, 2005).

TNF-$\alpha$ and IL-1 are early response mediators of lung inflammation and released by activated macrophages. They are initiators of cytokine networks and lead to neutrophil recruitment and chemotaxis (Lukacs and Ward, 1996). TNF-$\alpha$ and IL-1 play various actions including synergistic effects in inflammatory and immune response (Gulumian *et al.*, 2006). There are two forms, IL-1$\alpha$ and IL-1$\beta$, which play same role *in vitro*. IL-1 released from phagocytes, polynuclear white blood cells, and fibroblast plays an important role in lung fibrosis (Kolb *et al.*, 2001). TNF-$\alpha$ would be responsible for the initiation and perpetuation of the inflammatory reaction observed in the lung of patients with PMF. TNF-$\alpha$, which can directly induce fibroblast proliferation, could also trigger the production of mediators (Vanhee *et al.*, 1995). Blood levels of TNF-$\alpha$ and IL-1 were tended to increase in CWP (Vallyathan *et al.*, 2000). Schins and Borm (1995) reported that TNF-$\alpha$ was a predicted biomarker for progressive pneumoconiosis and its level correlated with severity of pneumoconiosis. Gulumian *et al.* (2006) reported that TNF-$\alpha$ was useful index for coal dust exposure and was useful biomarker for pneumoconiosis with progressive fibrosis in the lung. In this study, we found that the level of IL-1$\beta$ was not increased in the subjects with pneumoconiosis but positively correlated with x-ray profusion in pneumoconiosis patients with small opacity ($p < 0.05$). The level of TNF-$\alpha$ was higher in pneumoconiosis patients with large opacity than the control ($p < 0.05$), and positively correlated with x-ray profusion in the subjects with small opacity ($p < 0.01$). Theses results are in agreement with those of previous studies.

The level of IL-6 was increased in the patients with asthma and lung fibrosis (Reuben *et al.*, 2004). The level of IL-6 was increased in BALF or alveolar macrophages in CWP and associated with disease progression (Gosset *et al.*, 1991; Vallyathan *et al.*, 2000). Zhai *et al.* (2002) reported that the level of serum IL-6 correlated with pneumoconiosis classifications. In this study, the level of IL-6 tended to increase in the subjects with pneumoconiosis compared with the control but there was no significant difference ($p > 0.05$).

IL-8 is a structurally similar family of cytokine called chemokine, which demonstrates chemotactic activity for neutrophils. IL-8 is produced in response to proinflammatory stimuli. The accumulation of inflammatory leukocytes in the lung is hallmark of either acute or chronic pulmonary inflammation (Strieter *et al.*, 1993). The level of IL-8 was higher in the pneumoconiosis patients with small ($p < 0.05$) and large opacity ($p < 0.01$) than the control, and positively correlated with x-ray profusion in the subjects with small opacity ($p < 0.01$). The level of IL-8 in the severe group with the small opacity (ILO category II or III) was higher than that of the control ($p < 0.05$).

Many researchers have studied the relationship between proinflammatory cytokines, TNF-$\alpha$ and IL-1, and crystalline silica because TNF-$\alpha$ and IL-1 activate releasing of IL-6 or IL-8 (Gulumian *et al.*, 2006). In this study, TNF-$\alpha$ correlated with IL-6 ($r = 0.229$, $p < 0.01$), IL-8 ($r = 0.206$, $p < 0.05$), and IL-1$\beta$ ($r = 0.188$, $p < 0.05$).

MCP-1 plays an important role in the initial recruitment of cells such as lymphocytes and a small number of monocytes, and main role is as an activator and chemattractant of monocytes, leukocytes or lymphocytes (Toews, 2000). MCP-1 has been implicated in a variety of inflammatory diseases such as alveolitis and idiopathic pulmonary fibrosis (Loetscher *et al.*, 1994; Yoshimura *et al.*, 1989). Boitelle *et al.* (1997) reported that the level of MCP-1 was increased in BALF of CWP. MCP-1 regulates IL-1 and IL-6 (Biswas and Sodhi, 2002). In this study, the level of MCP-1 was higher in pneumoconiosis patients with large opacity than the control ($p < 0.05$), and positively correlated with x-ray profusion in the subjects with small opacity ($p < 0.01$). MCP-1 correlated with IL-6 ($r = 0.300$, $p < 0.01$) and IL-8 ($r = 0.255$, $p < 0.01$).

Although pneumoconiosis is the most prevalent lung disease showing decreasing of pulmonary function and emphysema (Schins and Borm, 1999), we found that measured cytokines were not correlated with the results of PFT. The reason of theses results can be explained that decreased PFT is the result of inflammation or fibrosis in the lung but cytokines are effects on current response of inflammation. Other possibilities may include that PFT is affected by the difference in the anatomy of the respiratory tract such as restriction of bronchus.

In accordance with previous studies, the level of blood cytokines showed significant difference among the categories of pneumoconiosis according to radiological findings, but the difference was not clear. For these reasons, although we discarded the subjects with the inflammation findings of liver and kidney in this study, the diagnostic specificity of measured blood cytokines is not satisfactory in the lung, because the levels of blood cytokines are affected by almost inflammatory response. It was necessary to monitor for the effects between cytokines and pneumoconiosis progression, including decreasing PFT and exacerbation of radiological findings. Future studies will be required to ascertain the cytokine profile that is present in pneumoconiosis patient using lung specific specimens such as BALF, exhaled breath condensate, or lung tissue.

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## REFERENCES

Ates, I., Suzen, H.S., Yucesoy, B., Tekin, I.O. and Karakaya, A. (2008). Association of cytokine gene polymorphisms in CWP and its severity in Turkish coal workers. *Am. J. Ind. Med.*, **51**, 741-747.

Becker, S., Soukup, J.M., Gilmour, M.I. and Devlin, R.B. (1996). Stimulation of human and rat alveolar macrophages by urban air particles: effects on oxidant radical generation and cytokine production. *Toxicol. Appl. Pharmacol.*, **141**, 637-648.

Biswas, S.K. and Sodhi, A. (2002). *In vitro* activation of murine peritoneal macrophages by monocytes chemoattractant protein upregulation of CD11b, production of proinflammatory cytokines, and the signal transduction pathway. *J. Interferon Cytokine Res.*, **22**, 527-538.

Boitelle, A., Gosset, P., Copin, M.C., Vanhee, D., Marquette, C.H., Wallaert, B., Gosselin, B. and Tonnel, A.B. (1997). MCP-1 secretion in lung from nonsmoking patients with coal worker's pneumoconiosis. *Eur. Respir. J.*, **10**, 557-562.

Brunzel, N.A. (2003). Clinical Chemistry-Concepts & applications: Renal function-Nonprotein, nitrogen compounds, function tests, and renal disease (Anderson, S.C. and Cockayne. S. Eds.), The McGraw-Hill Co. New York, pp. 373-399.

Brusasco, V., Crapo, R. and Viegi, G. (2005). Series "ATS/ERS Task Force: Standardisation of lung function testing". *Eur. Respir. J.*, **26**, 319-338.

Castranova, V., Vallyathan, V., Ramsey, D.M., McLaurin, J.L., Pack, D., Leonard, S., Barger, M.W., Ma, J.Y., Dalal, N.S. and Teass, A. (1997). Augmentation of pulmonary reactions to quartz inhalation by trace amounts of iron-containing particles. *Environ. Health Perspect.*, **105**, 1319-1324.

Dalal, N.S., Jafari, B., Peterson, M., Green, F.H. and Vallyathan, V. (1991). Presence of stable coal radicals in autopsied coal miners' lungs and its possible correlation to coal worker's pneumoconiosis. *Arch. Environ. Health*, **46**, 366-372.

Donalson, K., Brown, G.M., Brown, D.M., Robertson, M.D., Slight, J., Cowie, H., Jones, A.D., Bolton, R.E. and Davis, J.M.G. (1990). Contrasting brochoalveolar leukocyte in rat s inhaling coal mine dust, quartz, or titanium dioxide: effects of coal rank, airborne mass concentration, and cessation of exposure. *Environ. Res.*, **52**, 62-76.

Elias, J.A. and Zitnik, R.J. (1992). Cytokine-cytokine interactions in the context of cytokine networking. *Am. J. Respir. Cell Mol. Biol.*, **7**, 365-367.

Fitzgerald, S.P., Lamont, J.V., McConnell, R.I. and Benchikh, $E_L$.O. (2005). Development of high-throuput automated analyzer using biochip array technology. *Clin. Chem.*, **51**, 1165-1176.

Gosset, P., Lassale, P., Vanhee, D., Wallaert, C., Aerts, C., Voisin, C. and Tonne, A.B. (1991). Production of tumor necrosis factor alpha and interleukin-6 by human alveolar marcrophages exposed *in vitro* to coal mine dust. *Am. J. Resp. Cell Mol. Biol.*, **5**, 431-436.

Griwatz, U. and Seemayer, N.H. (1994). 29 P 13 Release of cytokines by quartz and coal mine dust exposed macrophages. *J. Aerosol Sci.*, **25**, 495-496.

Griwatz, U., Seemayer, N.H., Jung, B. and Dehnen, W. (1994). Cytokine production of human marcrophages induced by quartz and coal mine dusts: Tuberculosis and lung disease. *Int. J. Tuberc. Lung Dis.*, **75**, 103-104.

Gulumian, M., Borm, P.J.A., Vallyathan, V., Castranova, V., Donaldson, K., Nelson, G. and Murray, J. (2006). Mechanistically identified suitable biomarkers of exposure, effect, and susceptibility for silicosis and coal-worker's pneumoconiosis: a comprehensive review. *J. Toxicol. Environ. Health, Part B*, **9**, 357-395.

IARC (1997). IARC Monograph on the evaluation of the carcinogenic risk of chemicals to humans. In: Silica, some silicates, coal dust and para-aramid fibrils, Vol. 68. IARC Press, Geneva, Switzerland.

ILO (2002). Guidelines for the use of the ILO international classification of radiographs of pneumoconiosis. Geneva, International Labor Organization.

Ingram, L.R. (2003). Clinical Chemistry-Concepts & applications: Liver function (Anderson, S.C. and Cockayne, S. Eds.), The McGraw-Hill Co. New York, pp. 285-321.

Kolb, M., Margetts, P., Anthony, D.C., Pitossi, F. and Gauldie, J. (2001). Transient expression of IL-1 beta induces acute lung injury and chronic repair leading to pulmonary fibrosis. *J. Clin. Invest.*, **107**, 1529-1536.

Lasky, J.A., Oritz, L.A. and Brody, A.R. (2005). Lung injury-Mechanisms, pathophysiology, and therapy: Mediators and mechanisms in chronic lung injury and fibrosis (Notter, R.H., *et al.* Eds.). Taylor & Franics group, FL, pp. 175-226.

Loetscher, P., Seitz, M., Clark-Lewis, I., Baggiolini, M. and Moser, B. (1994). Monocyte chemotactic proteins MCP-1, MCP-2, and MCP-3 are major attractants for human CD4+ and CD8+ T lymphocytes. *FASEB J.*, **8**, 1055-1060.

Lukacs, N.W. and Ward, P.A. (1996). Inflammatory mediators, cytokines, and adhesion molecules in pulmonary and injury. *Adv. Immunol.*, **62**, 257-304.

Molloy, R.M., McConnell, R.I., Lamont, J.V. and Fitzgerald, S.P. (2005). (Review) Automation of biochip array technology for quality results. *Clin. Chem. Lab. Med.*, **43**, 1303-1313.

Morris, J.F., Koski, A. and Johnson, L.C. (1971). Spirometric standards for healthy nonsmoking adults. *Am. Rev. Respir. Dis.*, **103**, 57-67.

Perlman, D., Bitterman, P.B. and Wendt, C.H. (2005). Lung injury-Mechanisms, pathophysiology, and therapy: Chronic lung injury-Basic features and clinical relevance (Notter, R.H., *et al.* Eds.). Taylor & Franics group, FL, pp. 151-173.

Porter, D.W., Hubbs, A.F., Mercer, R., Robinson, V.A., Ramsey, D., McLaurin, J., Khan, A., Battelli, L., Mrumbaugh, K., Teass, A. and Castranova, V. (2004). Progression of lung inflammation and damage in rats after cessation of silica inhalation. *Toxicol. Sci.*, **79**, 370-380.

Prince, P., Boulay, M.E., Page, N., Desmeules, M. and Boulet, L.P. (2008). Induced sputum markers of fibrosis and decline in pulmonary function in asbestosis and silicosis: a pilot study. *Int. J. Tuberc. Lung. Dis.*, **12**, 813-819.

Razzaque, M.S. and Taguchi, T. (2003). (Review article) Pulmonary fibrosis: Cellular and molecular events. *Pathol. Int.*, **53**, 133-145.

Reuben, J.S., Guo, R.F. and Ward, P.A. (2004). Oxygen/Nitrogen radicals-Lung injury and disease: Mediators of lung inflammation-Role of reactive oxygen and nitrogen species (Vallyathan *et al.*, Ed.). Marcel Deckker, New York, pp. 91-110.

Schins, R.P.F. and Borm, P.J.A. (1995). Epidemiological evaluation of release of monocyte TNF-$\alpha$ as an exposure and effect marker in pneumoconiosis. *Occup. Environ. Med.*, **52**, 441-450.

Schins, R.P.F. and Borm, P.J.A. (1999). Mechanisms and mediators in coal dust induced toxicity: A review. *Am. Occup. Hyg.*, **43**, 7-33.

Shoemaker, D.A., Pretty, J.R., Ramsey, D.M., McLaurin, J.L., Khan, A., Teass, A.W., Castranova, V., Pailes, W.H., Dalal, N.S., Miles, P.R., Bowman, L., Leonard, S., Shumaker, J., Vallyathan, V. and Pack, D. (1995). Particle activity and *in vivo* pulmonary response to freshly milled and aged alpha-quartz. *Scand. J. Work Environ. Health*, **21**, 15-18.

Strieter, R.M., Standiford, T.J., Rolfe, M.W. and Kunkel, S.L. (1993). Cytokines of lung: Interleukine-8 (Kelly, J., Ed.). Marcel Deckker, New York, pp. 281-305.

Toews, G.B. (2000). Cytokines and pulmonary host defense against microbes: Cytokines in pulmonary disease-Infection and inflammation (Neson, S. and Martin, T.S., Eds.). Marcel Deckker, New York, pp. 1-17.

Tourmann, J.L. and Kaufmann, R. (1994). Biopersistence of the mineral matter of coal mine dusts in silicotic human lungs: is there a preferential release of iron? *Environ. Health Perspect.*, **102**, 265-268.

Ulker, O., Yucesoy, B., Demir, O., Tekin, I. and Karakaya, A. (2008). Serum and BAL cytokine and antioxidant enzyme levels at different stages of pneumoconiosis in coal workers. *Hum. Exp. Toxicol.*, **27**, 871-877.

Vallyathan, V. (2004). Oxygen/nitrogen radicals-lung injury and disease: Oxidative stress: antioxidant status in health and disease (Vallyathan *et al.*, Ed.). Marcel Deckker, New York, pp. 35-58.

Vallyathan, V., Goins, M., Lapp, L.N., Pack, D., Leonard, S., Shi, X. and Castranova, V. (2000). Changes in bronchoalveolar lavage indices associated with radiographic classification in coal miners. *Am. J. Respir. Crit. Care. Med.*, **162**, 958-965.

Vanhee, D., Gosset, P., Boitelle, A., Wallaert, B. and Tonnel, A.B. (1995). Cytokines and cytokine network in silicosis and coal workers' pneumoconiosis. *Eur. Respir. J.*, **8**, 834-842.

Wallaert, B., Lassalle, P., Fortin, F., Aerts, C., Bart, F., Fournier, E. and Voisin, C. (1990). Superoxide anion generation by alveolar inflammatory cells in simple pneumoconiosis and in progressive massive fibrosis of nonsmoking coal workers. *Am. Rev. Respir. Dis.*, **141**, 129-133.

Weber, S.L., Lapp, N.L., Vallyathan, V., Castranova, V., Shumaker, J. and Schwegler-Berry, D. (1996). Role of cytokines and mineral particle profile in the development of coal workers' pneumoconiosis as assessed by bronchoalveolar lavage. *Appl. Occup. Environ. Hyg.*, **11**, 923-927.

Yoshimura, T., Yuhki, N., Moore, S.K., Appella, E., Lerman, M.I. and Leonard, E.J. (1989). Human monocyte chemoattractant protein-1 (MCP-1). Full-length cDNA cloning, expression in mitogen-stimulated blood mononuclear leukocytes, and sequence similarity to mouse competence gene JE. *FEBS Lett*, **244**, 487-493.

Zhai, R., Liu, G., Ge, X., Bao, W., Wu, C., Yang, C. and Liang, D. (2002). Serum levels of tumor necrosis factor-alpha (TNF-$\alpha$), interleukin-6 (IL-6), and their soluble receptors in coal workers pneumoconiosis. *Respir. Med.*, **96**, 829-834.