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Original Article

The Serum Concentrations of YKL-40, IL-6, and TNF-α in Retired Workers Exposed to Inorganic Dusts

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Occupational long-term exposure to inorganic dusts may cause a variety of lung diseases such as pneumoconiosis and chronic obstructive pulmonary disease (COPD). Diagnosis of pneumoconiosis and COPD, however, is currently dependent on radiological findings and pulmonary test, which are both late diagnostic tools. Therefore, there is a need to identify novel biomarkers in pneumoconiosis and COPD. Hence, in this current study we investigated the serum concentrations of YKL-40, interleukin-6 (IL-6) and tumor necrosis factor-alpha (TNF-α) as biomarkers for pneumoconiosis and COPD in 161 retired male workers exposed to inorganic dusts. The serum concentration of YKL-40 was significantly increased with age, pneumoconiosis, and airflow limitation. The serum concentration of IL-6 was significantly higher in airflow limitation. These results suggest that serum concentration of YKL-40 is associated with age, pneumoconiosis, and airflow limitation. Also, serum concentration of IL-6 is associated with airflow limitation.

Key Words: YKL-40, IL-6, TNF-α, COPD, Pneumoconiosis

INTRODUCTION

Chronic occupational exposure to inorganic dusts such as coal and crystalline silica may cause a variety of interstitial lung diseases such as progressive massive fibrosis (PMF), coal workers pneumoconiosis (CWP), and chronic obstructive pulmonary disease (COPD), including parenchymal destruction (emphysema) and small airway disease (obstructive bronchiolitis) (Schins and Borm, 1999). Inhaled coal mine and silica dust may result in abnormal inflammatory response of the lung and leads to progressive airflow limitation that is characteristics of COPD (Vestbo et al., 2013). Diagnosis of pneumoconiosis is performed by radiological findings with occupational exposure history and pulmonary function test. Unfortunately, as current diagnostic tools of pneumoconiosis are only limited fibrosis in the lung which is usually irreversibly progressive, there have been some limitations in the detection of the early stage of pneumoconiosis. Therefore, it is necessary to study reliable and prospective biomarkers for pneumoconiosis before irreversible damage of the lung (Gulumian et al., 2006).

COPD is a complex disease involving more than airflow limitation and the "spill-over" of the inflammatory mediators into the circulation may result in systemic manifestations and comorbidities (Barnes and Celli, 2009). As in many patients with COPD have systemic inflammation, the levels of cytokines such as interleukin-6 (IL-6) and tumor necrosis factor-alpha (TNF-α) and acute phase proteins such as C-reactive protein (CRP) and YKL-40 are increased in the systemic circulation of COPD patients and abnormalities in circulating inflammatory cells such as lymphocytes have

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been reported (Agusti et al., 2003; Gan et al., 2004; Wouters et al., 2007).

YKL-40 belongs to the family of chitinase-like proteins and regulates mitogenesis, differentiation, and extracellular homeostasis in mammalian cells and has been associated with inflammation, tissue remodeling, fibrosis, and several malignancies (Johansen, 2006). In previous reports, serum concentrations of YKL-40 was up-regulated in patients with COPD (Létuvé et al., 2008) and associated with decline of lung function in the general population (Guerra et al., 2013).

Cytokines regulates various biological effects such as inflammation, metabolism, cell growth and proliferation, fibrosis, and homeostasis (Elias and Zitnik, 1992), Among these cytokines, IL-6 and TNF-α have been reported to be a prospective biomarkers to estimate the progression or exacerbation of pneumoconiosis and COPD (Di Francia et al., 1994; Razzaque and Taguchi, 2003; Vanhee et al., 1995; Yende et al., 2006). Although there were a few reports of the relationships between inflammatory mediators and occupational lung diseases such as pneumoconiosis and COPD (Lee et al., 2009; Lee et al., 2010), there was no report between YKL-40 in retired workers exposed to inorganic dusts in Korea. Therefore, there was a need to identify novel biomarkers in patients with pneumoconiosis and COPD who were exposed to chronic exposed to inorganic dusts. The present study was aimed to investigate the serum concentrations of YKL-40, IL-6, and TNF-α as biomarkers for pneumoconiosis and COPD in retired coal miners.

MATERIALS AND METHODS

Study Subjects

The study subjects contained 161 retired male workers exposed to inorganic dusts. We collected serum and stored at -80℃ until assay. Personal information including age, height, and weight as well as job history 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 Occupational Lung Diseases Institute.

Table 1. General characteristics of study subjects

Characteristics	N (%)	Mean*	SD*	Range
Age, yrs	161	62.8	8.0	38~82
~59	54 (33.5)			
60~69	75 (46.6)			
70~	32 (19.9)			
BMI, kg/m^2	161	23.8	2.8	15.4~30.5
Exposure period, yrs	161	18.0	8.7	2~46
~9	23 (14.3)			
10~19	77 (47.8)			
20~29	40 (24.8)			
30~	21 (13.0)			
Smoking, N	161			
Never	18 (11.2)			
Past	85 (52.8)			
Current	58 (36.0)			

*Arithmetic mean and arithmetic standard deviation
BMI, body mass index

Analysis of YKL-40, IL-6, and TNF-α

The concentrations of serum YKL-40 (MicroVue™ YKL-40 EIA, QUIDEL, USA), IL-6 (Human Interleukin-6 ELISA, BioVendor, Czech), and TNF-α (Human TNF-alpha ELISA, BioVendor, Czech) were analyzed by sandwich enzyme immunoassay.

Pulmonary function test

We carried out pulmonary function test in accordance with recommended guideline of ATS/ERS Task Force (Brusasco et al., 2005) by spirometry (Vmax22, Sensor-Medics, USA). We measured forced vital capacity (FVC), forced expiratory volume in one second (FEV_1), and FEV_1/FVC ratio.

Chest radiographs

chest radiographs were obtained and reviewed by the

pneumoconiosis review committee of Korea Workers' Compensation & Welfare Service. The radio opacity category of above 1/0 was defined as pneumoconiosis according to Internal Labor Office (ILO, 2002) classification.

RESULTS

General characteristics of study subjects

General characteristics of study subjects are shown in Table 1. In this study, 67 study subjects were classified as controls and 94 (41.6%) were classified as patients with pneumoconiosis (58.4%) (Table 2).

Serum concentrations of YKL-40, IL-6, and TNF-α according to general characteristics

The mean concentration of serum YKL-40 was significantly higher in subjects above 70 year (180.07 ng/mL) than that of less than 59 year (120.00 ng/mL) (P=0.020) and tended to increase with increment of years. There were no significant differences between mean concentrations of IL-6 and TNF-α in serum and general characteristics such as BMI, exposure period, and smoking status (Table 3).

Serum concentrations of YKL-40, IL-6, and TNF-α according to pneumoconiosis

The mean concentration of serum YKL-40 was significantly higher in subjects with pneumoconiosis (169.02 ng/mL) than that of controls (120.13 ng/mL) (P=0.004). However, there were no significant differences between mean concentrations of IL-6 and TNF-α and pneumoconiosis (Table 4).

Table 2. ILO categories of study subjects

ILO category	N	%	Profusion (N)
0 (Normal)	67	41.6	0/0 (54); 0/1 (13)
Pneumoconiosis	94	58.4	
Small opacity	78	48.4	
I	52	32.3	1/0 (19); 1/1 (19); 1/2 (14)
II, III	26	16.1	2/1 (13); 2/2 (8); 2/3 (4); 3/3 (1)
Large opacity	16	9.9	4A (13); 4B (3)

Table 3. Mean concentration of serum YKL-40 , IL-6, and TNF-α according to general characteristics

Characteristics		N	YKL-40 (ng/mL)	IL-6 (pg/mL)	TNF-α (pg/mL)
Age, yrs*	a. ~59	54	120.00 (2.10)	1.19 (2.82)	2.38 (402.75)
	b. 60~69	75	151.98 (2.11)	1.30 (3.20)	2.34 (143.89)
	c. 70~	32	189.07 (1.98)	1.27 (2.58)	2.36 (163.16)
			P=0.020	P=0.466	P=0.746
BMI, kg/m^{2}†	a. < 25.0	110	157.46 (2.19)	1.31 (2.81)	2.36 (119.00)
	b. ≥ 25.0	51	125.76 (1.91)	1.15 (3.32)	2.35 (1644.66)
			P=0.076	P=0.055	P=0.853
Exposure period, yrs*	a. ~9	23	152.19 (2.14)	1.32 (4.34)	2.44 (560.40)
	b. 10~19	77	154.24 (2.28)	1.24 (2.83)	2.36 (158.05)
	c. 20~29	40	134.57 (2.07)	1.20 (2.60)	2.35 (301.22)
	d. 30~	21	137.73 (1.57)	1.38 (3.06)	2.23 (153.92)
			P=0.784	P=0.652	P=0.156
Smoking*	a. Never	18	123.93 (2.18)	1.20 (2.67)	2.38 (384.24)
	b. Past	85	141.46 (2.00)	1.23 (2.75)	2.36 (338.98)
	c. Current	58	162.84 (2.25)	1.31 (3.33)	2.34 (96.91)
			P=0.327	P=0.648	P=0.922

*Calculated by ANOVA test (Tukey HSD test), geometric mean (geometric standard deviation)
†Calculated by student's t-test, geometric mean (geometric standard deviation)

Table 4. Concentration of serum YKL-40, IL-6, and TNF-α according to pneumoconiosis

	N	YKL-40 (ng/mL)	IL-6 (pg/mL)	TNF-α (pg/mL)
Controls	67	120.13 (2.17)	1.19 (3.01)	2.37 (284.60)
Patients with pneumoconiosis	94	169.02 (2.01)	1.31 (2.88)	2.35 (159.45)
		P=0.004	P=0.180	P=0.768

Control: ILO Classification 0/0 or 0/1, Pneumoconiosis: ILO classification 1/0 or more
*Calculated by student's *t*-test, geometric mean (geometric standard deviation)

Table 5. Concentration of serum YKL-40, IL-6, and TNF-α according to pulmonary function test

	N	YKL-40 (ng/mL)	IL-6 (pg/mL)	TNF-α (pg/mL)
$\%FEV_1/FVC$ ratio ≥ 70.0	95	129.48 (2.06)	1.17 (3.21)	2.35 (293.27)
$\%FEV_1/FVC$ ratio < 70.0	66	175.39 (2.13)	1.40 (2.71)	2.37 (127.50)
		P=0.011	P=0.012	P=0.649

$\%FEV_1/FVC$ ratio < 70.0: Airflow limitation
* Calculated by student's *t*-test, geometric mean (geometric standard deviation)

Serum concentrations of YKL-40, IL-6, and TNF-α according to pulmonary function test

The mean concentration of serum YKL-40 was significantly higher in subjects with airflow limitation (less than $\%FEV_1/FVC$ ratio < 70) (175.39 ng/mL) than that of above 70% (129.48 ng/mL) (P=0.011). Also, the mean concentration of serum IL-6 was significantly higher in subjects with airflow limitation (1.40 pg/mL) than that of above 70% (1.17 pg/mL) (P=0.012). There was no significant difference between mean concentration of TNF-α and FEV_1/FVC ratio (Table 5).

DISCUSSION

YKL-40 is expressed and secreted by activated monocytes (Hashimoto et al., 1999), late stage of macrophages (Renkema et al., 1998), vascular smooth muscle cells (Nishikawa and Millis, 2003), and cancer cells (Johanson et al., 2006). YKL-40 is important role in cell proliferation and differentiation (Brochner et al., 2011), inflammation (Kawada et al., 2012), extracellular tissue remodeling (Lee et al., 2011), and protection against apoptosis (Lee et al., 2009). Plasma YKL-40 levels correlated with age in both sexes (Johansen et al., 2009). High levels of YKL-40 in circulation and bronchoalveolar lavage fluid (BALF) is associated with asthma (Chupp et al., 2007), COPD (Letuve et al., 2008), and idiopathic pulmonary fibrosis (IPF) (Korthagen et al., 2011). Furthermore, YKL-40 is negatively correlated to $\%FEV_1$ predicted in patients with COPD and asthma (Sakazaki et al., 2011). In recent study, high plasma YKL-40 is associated with mortality in patients with moderate and very severe COPD, suggesting a role for as a potential biomarker of mortality in this patient group (Holmgaard et al., 2013). In present study, high serum concentrations of YKL-40 showed significant association with age, pneumoconiosis, and airflow limitation, a main characteristics of COPD and tended to increase with age.

Pneumoconiosis results from chronic inhalation to inorganic dusts and is characterized by a pulmonary fibrosis in the lung and irreversible lung damage (Schins and Borm, 1999). Inhaled inorganic dusts may induce release of proinflammatory cytokines such as TNF-α (Lasky et al., 2005) and IL-6 stimulates production of acute-phase protein such as YKL-40, but not TNF-α (Ferrari et al., 2013). Thus, IL-6 could be an upstream activator of YKL-40 independent of IL-6 (Nielsen et al., 2011). The concentration of IL-6 was increased in BALF and alveolar macrophages in patients with pneumoconiosis and associated with disease progression (Gosset et al., 1991; Reuben et al., 2004; Vallyathan

et al., 2000; Zhai et al., 2002). Although pneumoconiosis is strongly related with serum IL-6 and TNF-α, we found that measured serum cytokines were not related with the results of pneumoconiosis in this study, high serum concentrations of IL-6 showed significant association with patients with airflow limitation.

This study has several limitations. One is that mean age of study subjects was over 60 years. Thus, systemic inflammation may be still in existence results from other systemic comorbidities such as diabetes, hypertension, and rheumatoid arthritis. It was necessary to compare with normal controls without occupational history of inorganic dusts and comorbidities.

In conclusion, high serum concentration of YKL-40 is associated with age, pneumoconiosis, and airflow limitation. Also, high serum concentration of IL-6 is associated with airflow limitation. Further studies will be required to investigate the potential serum acute-phase proteins and cytokines in patients with pneumoconiosis and COPD using lung specific specimens such as BALF, exhaled breath condensate, and lung tissue.

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