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Plasma levels of TNF-α and MMP-9 in patients with silicosis

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Abstract. – OBJECTIVE: Silicosis is usually recognized at later stages of the disease, and early biomarkers for silicosis will be useful for timely diagnosis. We aimed at examining plasma levels of TNF-α and MMP-9, and correlation between these, in patients with different stages of silicosis in order to test suitability of these inflammatory factors as early biomarkers for silicosis.

PATIENTS AND METHODS: TNF-α and MMP-9 were quantified by ELISA in plasma specimens from 30 healthy individuals (control group), 28 individuals exposed to silica dust but without clinical disease, and 30 patients with silicosis.

RESULTS: Plasma levels of TNF-α and MMP-9 were increased in individuals exposed to silica dust ($p < 0.05$ vs. control individuals) and were further elevated in patients with silicosis ($p < 0.05$ vs. control individuals and individuals exposed to silica dust). There was a significant correlation between plasma levels of TNF-α and MMP-9 both in individuals exposed to silica dust ($r = 0.696$, $p < 0.01$) and patients with silicosis ($r = 0.768$, $p < 0.01$).

CONCLUSIONS: Plasma levels of TNF-α and MMP-9 are increased prior to development of clinically recognized silicosis, suggesting that these biomarkers are involved in the onset and development of silicosis. Combined detection of TNF-α and MMP-9 may be useful for early diagnosis of silicosis.

Key Words:
Silicosis, Plasma, TNF-α, MMP-9, Correlation.

Introduction

Silicosis is caused by long-term inhalation of free silica (SiO_2) dust. This disease is the most common and severe forms of pneumoconiosis. The main pathological features are macrophage-dominated pulmonary alveolitis, silicotic nodules, and lung fibrosis caused by silica dust[1]. The role of macrophages in silicosis development have been examined in some studies[1,2], but the pathogenesis of silicosis in general remains unclear. In most patients, clinical diagnosis is done when chest X-ray abnormalities have already developed. Unfortunately, at that stage, the lesions become irreversible. Therefore, early diagnosis of silicosis is of great importance[2]. In this study, we examined plasma levels of Tumor Necrosis Factor (TNF)-α and Matrix Metalloproteinase (MMP)-9, and correlation between these, in individuals exposed to silica dust to test potential suitability of these factors for early diagnosis of silicosis.

TNF-α is an important cytokine initiating inflammatory responses. Studies demonstrated increased production of TNF-α in blood mononuclear cells from patients with silicosis[3].

MMP-9 regulates cell differentiation and proliferation, and MMP-9 levels are up-regulated by TNF-α. Thus, both cytokines may be involved in the development of silicosis[4].

Patients and Methods

Patients and Study Groups

Control individuals included 30 employees who had regular physical examinations. Individuals exposed to silica dust (n = 28) have been in contact with it at work for more than 1 year. These were 28 workers from a machine factory and were recruited from Lishui City in Zhejiang Province. This city is situated in a mountainous

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region, and the population has a high incidence rate of silicosis. Silicosis group comprised 30 patients with clinical disease, including 8 with silicosis stage I, 12 with stage II, and 10 with stage III. The patients were both in- and outpatients. The majority of the patients with silicosis originated from Jinyun and Qingtian Counties of Zhejiang Province, where most people are engaged in stone industry. All study individual were male. Patients with hypertension, diabetes mellitus, diseases of the heart, kidney, liver or blood, rheumatoid disease, immune system disease or tumours were excluded from the study.

None of study individuals were not engaged in occupations associated with exposure to radiation and toxic substances, and were not chronically exposed to pesticides. Study individuals had normal digestion and absorption functions, and did not take vitamin C, or preparations derived from ginkgo leaf or tea polyphenols, or antioxidant drugs within one month prior to the study.

The average age of control individuals was (mean ± SD) 48.66 ± 8.02 years. The individuals exposed to silica dust were aged 50.76 ± 9.40 years. The average age of patients with silicosis was 52.29 ± 10.42 years. There were no significant differences in age among study groups.

Diagnosis of Silicosis

The history of exposure to silica dust was gathered for the diagnosis of silicosis. In addition, posterior-anterior chest X-ray exam was conducted using a high-kilovar X-ray machine according to the National Criteria for X-ray Diagnosis of Pneumoconiosis in China[5]. The integrated diagnosis of silicosis was made by a pneumoconiosis diagnosis group including exposure history, chest radiographs, and silicosis prevalence in the organization where individuals worked. The diagnosis respected the "Diagnostic Standard for Pneumoconiosis"[6]. Silicosis was classified in three stages[7]:

Pneumoconiosis, stage I: round small shadows of grade I intensity distributed at least in one segment of either lung; the diameter of each shadow was at least 2 cm; alternatively, patients could present with the irregular shape small shadows of grade I intensity, distributed in more than two lung segments. Pneumoconiosis, stage I+: intensity of small shadows increased significantly, but the intensity or distribution range did not fulfil yet the definitions of pneumoconiosis, stage II.

Pneumoconiosis, stage II: small round or irregular shadows of grade II distributed in more than

Table I. Demographic and clinical characteristics of study individuals.

Study groups (numbers)	Age (years)	Average onset time (years)	The average dust exposure time (years)	Respiratory symptoms			Pulmonary function
				Cough and (number, %)	Chest expectoration tightness and shortness of breath (number, %)	(0, 0%)	
Control individuals (30)	48.6 ± 8.02	-	-	0, 0%	0, 0%	0, 0%	Normal
Individuals exposed to silica dust (28)	50.7 ± 9.40	-	1.57	11, 36%	0, 0%	0, 0%	Borderline abnormal
Pneumosilicosis, stage I (8)	51.4 ± 10.20	7.25	5.89	6, 75%	4, 50%	0, 0%	Mild to moderate airflow limitation and restrictive changes
Pneumosilicosis, stage II (12)	49.8 ± 10.33	12.14	6.78	11, 91.2%	8, 66.7%	3, 25%	Moderate to severe airflow limitation and restrictive changes
Pneumosilicosis, stage III (10)	52.29 ± 10.42	15.25	8.15	10, 100%	10, 100%	7, 70%	Severe airflow limitation and restrictive changes

Footnote: Data are presented as mean ± SD or absolute numbers (%).

Table II. Plasma concentrations of TNF-α and MMP-9 in study individuals.

Study groups	Numbers	TNF-α, pg/ml	MMP-9, ng/ml
Control individuals	30	23.13 ± 12.10	32.64 ± 12.56
Individuals exposed to silica dust	28	39.31 ± 14.16*	68.75 ± 15.63*
Patients with silicosis	30	52.75 ± 21.13#&	108.79 ± 17.23#&

Footnote: Data are presented as mean ± SD. *$p < 0.01$ vs. control individuals, #$p < 0.05$ vs. patients exposed to silica dust, &$p < 0.01$.

four pulmonary segments; shadows of grade III distributed in four pulmonary segments. Pneumoconiosis, stage II+: intensive small shadows of grade III, distributed in more than four lung segments, or a big shadow of less than grade III.

Pneumoconiosis, stage III: big shadow with the length measuring at least 2 cm and width at least 1 cm. Pneumoconiosis, stage III+: single large shadow area or sum of multiple large shadows bigger than the right lung area.

Reagents and Instruments

The TNF-α and MMP-9 ELISA kits were purchased by Biosource (Nivelles, Belgium). The analyses were made using the automatic biochemical analyzer (Beckman, Brea, CA, USA).

Blood Sampling for TNF-α and MMP-9 Analyses

Fasting peripheral venous blood (5 ml) was sampled early in the morning. Blood sample was collected in a dry tube containing EDTA and immediately subjected to gentle mixing and centrifugation (2500 rom; 10 min). Plasma was cryopreserved at -80º C pending analysis.

Statistical Analysis

The statistical software SPSS 11.5 (IBM, Chicago, IL; USA) was used for statistical analysis. Data are expressed as mean ± SD. A single-way ANOVA was used to test for statistical differences. The differences at $p < 0.05$ were considered statistically significant.

Results

The demographic and clinical characteristics of study individuals are shown in Table I.

Plasma levels of TNF-α and MMP-9

Plasma levels of inflammatory biomarkers were significantly higher in individuals exposed to silica dust and patients with silicosis, compared with control individuals (Table II). Furthermore, plasma levels of both biomarkers in patients with silicosis were significantly higher than in individuals exposed to dust (Table II).

Correlation Between TNF-α and MMP-9

As shown in Table 3, there was a significant and positive correlation between TNF-α and MMP-9 in both individuals exposed to silica dust ($r = 0.696$, $p < 0.01$) and patients with silicosis ($r = 0.768$, $p < 0.01$; Table III).

Discussion

Silicosis has the highest incidence in workers exposed to dust. There is no current effective treatment against silicosis. The latest survey shows that the shortest exposure time for silicosis in formal employees of a state-owned large coal enterprise is about 25 years. The incidence rate of this disease is 0.89%. However, the shortest exposure time for silicosis in migrant worker groups of small and medium-sized enterprises is about 1.5 years (average of 6.69 years[8]), which is consistent with observations made in our city. This may be caused by inadequate protection from dust in medium-sized coal enterprises and protection negligence from workers.

Silicosis progresses rapidly; thus, patient prognosis is unfavourable when the patients are diagnosed with this disease. A variety of cytokines are involved in the onset and progression of silicosis. For example, TGF-β_1 and TNF-α are important cytokines involved in the pathogenesis of fibrosis. These cytokines stimulate the synthesis and deposition of extracellular matrix, and collagen synthesis in fibroblasts, thereby, promoting development of fibrosis[9]. TNF-± is secreted by mononuclear macrophages, fibroblasts, lymphocytes, and smooth muscle cells[10]. The major role of TNF-α in silicosis is to induce influx of inflammatory cells, promote secretion of other cytokines, potentiate fibroblast prolifer-

Table III. Correlation between plasma levels of TNF-α and MMP-9.

Study groups	Numbers	TNF-α (pg/ml)	MMP-9 (ng/ml)	r	p
Individuals exposed to silica dust	30	39.31 ± 14.16	68.75 ± 15.63	0.696	<0.01
Pneumosilicosis, stage I	8	47.15 ± 17.21	94.57 ± 16.14	0.712	<0.01
Pneumosilicosis, stage II	12	52.13 ± 19.24	106.4 ± 17.19	0.745	<0.01
Pneumosilicosis, stage III	10	61.52 ± 23.35	109.1 ± 18.21	0.768	<0.01

ation and collagen deposition. TNF-α up-regulates expression of several fiber-forming factors (e.g., fibronectin and collagen) and induces aggregation of platelets in the lungs. Activated platelets contribute to fibrosis by releasing TGF-β, platelet-derived growth factor, and other pro-fibrotic cytokines. Thus, TNF-α plays a major role in the induction of chemokine production by silica dust. Silica, diamond and asbestos dusts up-regulate TNF-α in alveolar macrophages[11]. Also, instillation of quartz dust into mice trachea was shown to increase expression of TNF-α mRNA[12]. Corroborating this, anti-TNF-α antibody prevented up-regulation of chemotactic factor TNF-α induced by silica dust[13]. Thus, chemotactic response to silica dust is not only due to oxidant exposure but also involves production TNF-α.

In our study, plasma levels of TNF-α were significantly higher in patients with silicosis compared with control individuals, indicating that TNF-α is involved in the development of silicosis. We speculate that TNF-α is highly expressed in workers with silicosis because of a long-term exposure to silica dust in the absence of proper protective measures, which leads to fibrosis. This is supported by the fact that workers exposed to silica dust and who have not yet developed silicosis exhibit plasma levels of TNF-α higher than healthy controls, but lower than patients with silicosis. Therefore, TNF-α levels can be used as an early biomarker of silicosis.

Besides afore-mentioned chemokine-promoting role of TNF-α, this cytokine also up-regulates expression and activates MMP-9[17]. MMP and their tissue inhibitors (TIMP) are secreted by effector cells in fibrosis and are important for the synthesis and degradation of the extracellular matrix[14]. The substrate for MMP-9 is mainly the type IV collagen, which is an important component of the basement membrane in alveolar wall. Damage to alveolar epithelial cells and basement membrane of the alveolar wall are key events in the development of fibrosis[15]. After exposure to silica dust, fibroblasts in pulmonary mesenchyma contribute to the damage in basement membrane of pulmonary alveoli by expressing MMP-9[16].

Conclusions

We think that TNF-α plays an important role in the development of pulmonary fibrosis with silicosis, and the levels of TNF-α and MMP-9 are associated with the time of exposure to silica dust. Increase in the levels of TNF-α and MMP-9 in individuals exposed to silica dust but without clinical disease indicates that TNF-α and MMP-9 can be used as biomarker of early silicosis. Since TNF-α can be elevated during processes with increased inflammatory responses, such as viral, bacterial, or parasite infections, trauma, this biomarker can easy be false positive[18]. However, MMP-9 is a key enzyme in lung interstitial fibrotic processes, and its elevation has a higher specificity. Both TNF-α and MMP-9 can be measured by ELISA which is a simple and reliable detection method. Combined detection of TNF-α and MMP-9 can increase their sensitivity and specificity as biomarkers for early silicosis.

Conflict of Interest

The Authors declare that they have no conflict of interests.

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