

Full Length Research Paper

Study on the serum oxidative stress status in silicosis patients

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To determine whether oxidative-stress damage play an important role in the mechanism of silicosis, the oxidative stress parameters were investigated in silicosis patients and controls group. 128 silicosis patients and 130 healthy controls were included. The serum superoxide dismutase (SOD) activity and the levels of malonyldialdehyde (MDA) and glutathione (GSH) were analyzed. The levels of GSH and MDA in silicosis patients were significantly higher than those of the controls group. SOD activity was higher in the silicosis group than that in the controls ($p < 0.05$) except for III stage. None of the 3 variables examined were associated with the age among both the controls and silicosis patients. The GSH level and SOD activity significantly declined over a prolonged disease period, while MDA levels remained largely unaffected by the disease duration. These results confirmed the role of oxidative stress in the mechanism of silicosis. Therefore, effective antioxidant therapy for inhibiting oxidative stress may be a therapeutic option in silicosis.

Key words: Silica, silicosis, superoxide dismutase, glutathione, malondialdehyde.

INTRODUCTION

Silicosis is an occupational lung disease caused by inhalation of dust containing crystalline silica (Laney et al., 2010). Currently, the effect of oxidative damage in the mechanism of silicosis is of great interest to the research community (Barrett et al., 1999; Zhang et al., 2000; Park and Park., 2009). Oxidative stress can be induced either by an excessive production of free radicals or by loss of antioxidant defense mechanisms (Halliwell, 1994; Veskoukis et al., 2009). Free radicals generated either by the surface activity of crystalline silica or by the inflammatory cells with the alveolar macrophage are invoked by crystalline silica (Vallyathan et al., 1988; Balduzzi et al., 2004). Many researches reported the potential effect of oxidative stress in silicosis model (Cho et al., 1999; Porter et al., 2002), but few data are available related to the correlation between disease duration and the oxidative stress parameters in so many silicosis patients. Therefore, the aim of this study was to

examine the anti-oxidative status of patients with silicosis to help clinicians to further delineate the role of oxidative-stress parameters of patients with silicosis and provide novel clues for discerning the pathogenesis of the disease, as well as aiding in providing novel therapeutic strategies (Gulumian, 1999). The serum superoxide dismutase (SOD) activities, glutathione (GSH) and malondialdehyde (MDA) levels were analyzed in silicosis patients and in control group.

MATERIALS AND METHODS

Study population

Silicosis is diagnosed based on a history of exposure to SiO₂ dust, relevant epidemiological data from industrial hygiene surveys, classical presentation on dorsoventral chest radiography, clinical and laboratory examinations and the exclusion of other similar pulmonary diseases. According to the Chinese National Diagnostic Criteria of Pneumoconiosis GB5906-86 (Ministry of Health), Stage 0: X-ray shows no pneumoconiosis (0) or x-ray presentation is not sufficient for classifying as Stage I (0*). Stage I: Profusion Grade 1 small rounded opacities with distributions in at least two lung zones, each with an area of diameter not less than 2 cm or profusion grade

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Table 1. Clinical characteristics of silicosis patients control subjects.

Characteristic	Control group	Silicosis group
Age (years)	62.9 ± 12.3	61.3 ± 13.0
Working duration (years)	28.9 ± 5.7	24.8 ± 5.3
Exposure duration (years)	0	21.8 ± 8.3
Smoking (%)	10 (7.7)	6 (4.7)
Total cholesterol (mmol/l)	4.13 ± 1.12	4.47 ± 1.49
ALT (U/L)	29.8 ± 10.6	32.5 ± 12.9
Creatinine (µmo/l)	67.8 ± 12.4	61.2 ± 10.9
Glucose (mmol/l)	5.03 ± 0.89	5.27 ± 1.11

1 small irregular opacities with distributions in at least two lung zones (I); or small opacities are obviously more but either the profusion or distribution is not sufficient for classifying as II (I⁺). Stage II: Profusion Grade 2 small rounded or irregular opacities with distributions in more than four lung zones or profusion grade 3 small opacities with distributions reaching four lung zones (II); or profusion grade 3 small opacities with distributions in more than four lung zones or large opacities not sufficient for classifying as III (II⁺). Stage III: Large opacities of length not less than 2 cm and width not less than 1 cm (III); or single large opacities with an area or multiple large opacities with total area greater than the right upper lung zone (III⁺). 128 male patients who had been diagnosed with silicosis were admitted to the occupational disease hospital of the state grid, with a mean age of 61.3 years. Among those patients, 29 patients were in the category of stage 0, 68 were in stage I, 21 were in stage II and 10 were in stage III. The control group included 130 men (their mean age was 62.9 years) without any history of SiO₂ dust exposure and with no evidence of heart, liver, kidney or lung disease. Information on demographic characteristics, lifestyle and occupational features were collected by means of a questionnaire. Some data of the investigated groups are given in Table 1.

Ethics statement

This research programme was approved by the Ethics Committee of the Occupational Disease Hospital of State Grid and was in accordance with the principles of the declaration of Helsinki. Written consent forms were obtained from all subjects.

Sample collection

We collected 7 ml of peripheral venous blood from the fasting subjects. After coagulation, the blood samples were centrifuged at 3000 r/min for 5 min, serum was collected and the serum samples were stored at -80°C for further experiments. Complete blood analysis was done to determine the serum SOD activities, GSH and MDA levels, additionally, routine biochemical parameters were analyzed.

Biochemical assays

Serum GSH was estimated by the dithiobisnitrobenzoic acid method reported by Thomas and Skrinska (1985), which is based on the reaction of GSH with the dithiobisnitrobenzoic acid to form yellow compounds that absorbs at 420 nm.

The serum SOD activity was determined spectrophotometrically by the methods of Beauchamp and Fridovich (1971), in which the

ratio of auto-oxidation rates of the samples was determined. The activity of SOD was calculated in terms of units defined as the amount of SOD that showed 50% inhibition of the reduction of nitroblue tetrazolium (NBT).

The MDA levels were measured spectrophotometrically by the methods of Uchiyama and Mihara (1978). Briefly, MDA was allowed to react with thiobarbituric acid that yielded red-colored products, which were spectrophotometrically quantified by measuring the maximal-absorption peaks at 532 nm.

Statistical analysis

Values were expressed as means ± SD; the statistical analyses were performed using SPSS statistical software 11.0. The Wilcoxon-Mann-Whitney non-parametric test was used to analyze the differences between groups. Relationships between variables were tested using Pearson's correlation analysis. P values of less than 0.05 were regarded as significant.

RESULTS

Table 1 summarizes the baseline characteristics among the different groups. There were no significant differences in age, working duration smoking, total cholesterol, ALT, creatinine and glucose between silicosis group and control group.

As shown in Table 2, the serum GSH and MDA levels were higher among the silicosis group in comparison to the controls ($p < 0.05$). SOD activity was higher in the silicosis group than that of controls except in stage III ($p < 0.05$).

Interestingly, none of the 3 variables examined (levels of serum GSH, MDA and SOD activity) were associated with the age among the controls and the silicosis patients (Figure 1, $p > 0.05$). However, the serum GSH and SOD levels decreased significantly with an increase in the disease duration, while MDA levels remained largely unaffected by the disease duration (Figure 2).

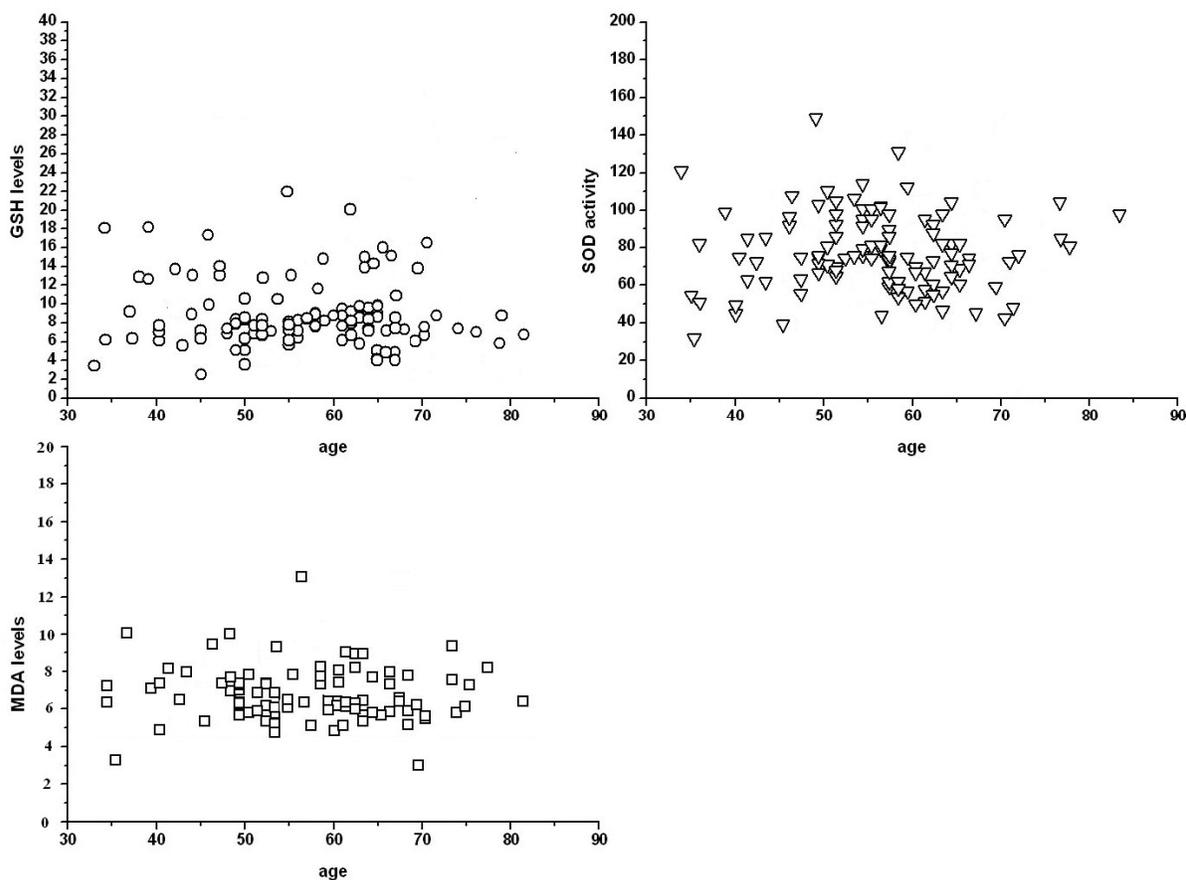
DISCUSSION

Inhalation of silica dust causes a systemic injury that is mainly characterized by interstitial fibrosis within the lung

Table 2. Comparison of levels of serum oxidation markers (SOD, GSH and MDA) to the control levels and between different stages of silicosis (±s).

Group	GSH level (mg/l)	SOD activity (U/ml)	MDA level (nmol/ml)
Control	4.4±1.6	72.6±16.4	4.1±1.4
O stage	11.7±2.5*	102.1±19.0*	8.6±2.1*
I stage	14.6±2.8*	96.1±20.0*	9.4±2.3*
II stage	9.6±2.2*	86.6±22.4*	10.3±2.4*
III stage	9.4±2.7*	78.9±23.2	10.5±2.7*

*p < 0.05 vs. control group.

**Figure 1.** Dot plot showing associations between serum GSH, MDA, SOD levels and age in silicosis patients.

(Castranova et al., 2002; Birk et al., 2010). Several reports indicate that, ROS and their reactions with lung epithelial cells and alveolar macrophages participate in the development of pulmonary disease in the silicosis (Kuвано, 2008). In normal state, there is a dynamic redox balance between oxidants and antioxidant defense mechanisms. Silica may induce oxidative stress leading to generation of free radicals and alterations in antioxidant and scavengers of oxygen free radicals (OFRs) (Shi et al., 1995). In *in vivo* experiment, exposure

to a crystalline silicon dioxide particle resulted in an increased generation of ROS with enhanced levels of oxidative enzymes and lipid peroxidation in rat lung (Vallyathan et al., 1997). GSH is composed of glutamate, glycine and cysteine and is the major non-protein sulfhydryl compound present in tissue. Boehme et al. (1992) showed that, glutathione was released by rat pulmonary alveolar macrophages in response to silica particle *in vitro* study. There are few studies related to oxidative stress parameters of blood in silicosis patients.

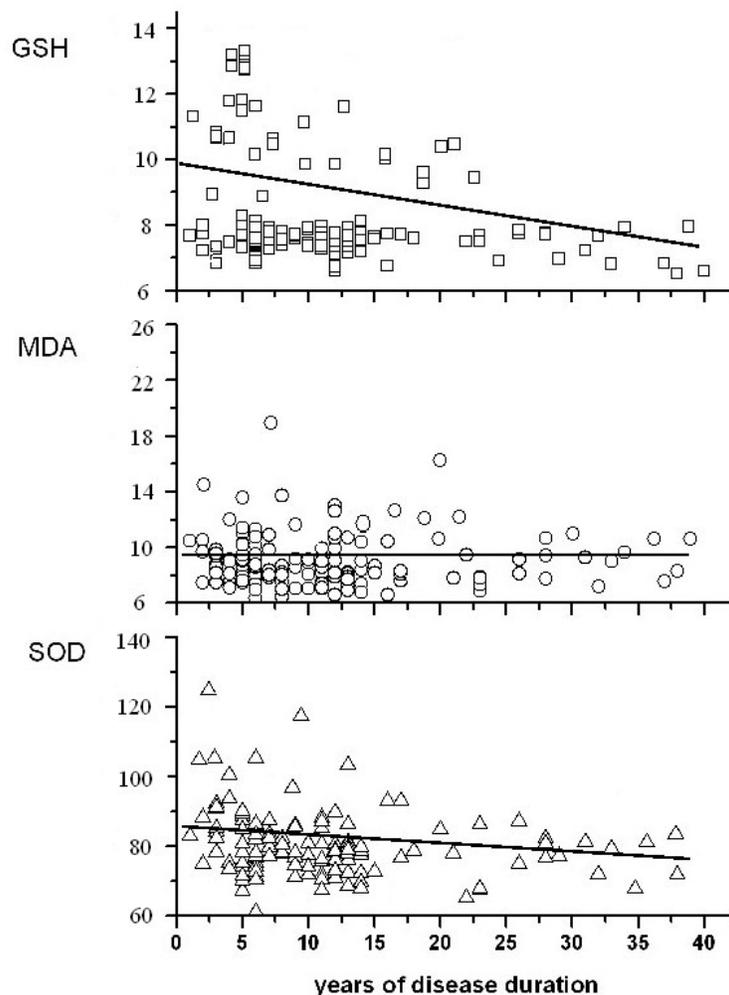


Figure 2. Dot plots illustrating the associations between serum GSH, MDA and SOD levels and disease duration.

Although, the GSH levels in red blood cells of silicosis patients have been reported to be higher, but no differences in red blood SOD or plasma lipid peroxidation were observed between silicosis patients and control subjects (Borm et al., 1986). On other hand, Orman et al. (2005) showed a decreased erythrocyte GSH levels and an increased plasma MDA levels in workers with cement dust-exposure silicosis. Our findings indicated that the serum GSH and MDA levels were higher among the silicosis group in comparison to the controls ($p < 0.05$). SOD activity was higher in the silicosis group than that of the controls except for stage III ($p < 0.05$). This suggests that, exposure to SiO_2 dust stimulated the body-defending antioxidant mechanisms. But the elevated concentration of MDA indicated that, the stimulation of the body's antioxidant agents failed to cope with the overproduction of the reactive oxygen species in the silicosis patients. Furthermore, the research seem to indicate the concentration of antioxidants which first increased then drops during the different stages of silicosis.

Nevertheless, large-scale clinical trials are still required to elucidate the role of antioxidants.

None of the 3 variables examined were associated with the age among the controls and silicosis patients in our study. The erythrocyte SOD activity was found to be negatively related to the age in the healthy subjects (Ceballos-picot et al., 1992). Also, the GSH content of blood lymphocytes was significantly lower when compared with age in healthy controls (Van et al., 1998). In agreement with our results, Kamal et al. (1989) reported that, neither age nor duration of exposure was related to the MDA levels among the workers exposed to silica dusts. Contradictory to our finding, Kamal et al. indicated that, neither SOD nor MDA level was related to age among the asbestos exposed workers and the controls (Kamal et al., 1992). We found that, the GSH and SOD levels were negatively correlated with the disease duration. According to the free radical theory proposed to explain the mechanism of silicosis, lung alveolar macrophages were activated then released

various reactive oxidative species (ROS) in long-lasting exposure to silica dust (Shi et al., 1988). ROS continually consumed the antioxidant agents present in the body, thereby indicating the failure of the total antioxidant defense mechanism.

Taking collectively, this study confirmed the premise that, oxidative stress may be an important event in silicosis patients. Hence, it can be concluded that detection serum levels of SOD, GSH and MDA is helpful to interpret the oxidative stress effect on the pathogenesis of silicosis. Moreover, the use of antioxidants may be beneficial in silicosis treatment; certainly more clinical trails must be conducted to elucidate the role of antioxidants.

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