

**Date: 19<sup>th</sup> March 2009**

**Final Report: The Role of Oxidants and Antioxidants in the Pathogenesis of Silicosis**

**Overall objective:**

The main objective is to study the role of oxidants and antioxidants in the pathogenesis of silicosis provided by the opportunity of the study “Early lung cancer detection with sputum examination and autofluorescence bronchoscopy in subjects with pneumoconiosis”.

**Specific aims:**

1. To determine the oxidant status in the lung by measuring the levels of plasma 8-isoprostane in patients with silicosis and controls;
2. To determine the status of systemic antioxidant status by measuring the enzyme activity levels of SOD, catalase, glutathione peroxidase (GPx) and compare the levels with healthy controls matched for smoking;
3. To measure the plasma levels of IL-8, an inflammatory cytokine, in patients and controls;
4. To measure the levels of pro-inflammatory cytokines and fibrogenic factors such as MMP-2, MMP-9 and TGF- $\beta$ 1 in the plasma and to correlate these levels with the degree of oxidative stress;
5. To correlate the levels of these antioxidants, oxidants, and the level of pro-inflammatory cytokines, fibrogenic factors and inflammatory mediators in the peripheral blood and in BAL with the severity of silicosis as measured by lung function.

**Summary and Comments:**

- Among patients with silicosis with atypical sputum referred for autofluorescent bronchoscopy, one was found to have carcinoma-in-situ and one had severe dysplasia. The one with carcinoma in situ was treated with cryotherapy while the one with severe dysplasia was diagnosed to have lung cancer later. No one in the smoking controls was found to have lung cancer in this study. This study shows that the method of autofluorescent bronchoscopy is also highly suitable for early detection of lung cancer in patients with silicosis where the cancers are situated in the main bronchi mostly as a result of heavy smoking.
- In analyzing the data, we felt that as both groups of subjects were smokers, it would be interesting to compare with the results of those of the healthy nonsmokers as well. This was not part of the protocol and no funds were requested for this purpose. We do have some existing data of nonsmoking healthy subjects that we have assayed in the past. This group of nonsmokers was younger and half of them are females. However, if we were to choose only males and smokers, the number would be much smaller.
- There is evidence of oxidative stress in patients with silicosis as shown by the elevated plasma levels of 8-isoprostane, which is a unique product of lipid peroxidation and a reliable marker of oxidant injury both in vitro and in vivo (32). All patients with silicosis in this study were also smokers and oxidative stress is well established among smokers. In this study, patients with silicosis not only had the highest plasma 8-isoprostane level but the level was significantly higher than the smoking controls consistent with the observation that silica also causes oxidative injury.

- Levels of antioxidants were not different between patients with silicosis and smoking controls.
- The levels of IL-8, which is an inflammatory cytokine, were not increased in the plasma in both patients with silicosis and smoking controls compared with nonsmokers.
- In this study we found that plasma levels of TGF- $\beta$ 1 were significantly elevated compared with nonsmokers but not significantly different from smoking controls. It is well known that smoking also causes increase in level of TGF- $\beta$ 1 (33). Thus the increase in TGF- $\beta$  is likely due to smoking and not to the effect of silica inducing fibrosis in this study. It is possible that most patients in this study has mild simple silicosis with little fibrosis as demonstrated by the fact that the lung function was within normal limits in a number of patients.
- In this study, MMP-2 activity levels were higher in silicosis patients compared with controls. As we have not yet assayed MMP-2 and MMP-9 activities in plasma of nonsmokers, the significance of the higher levels of MMP-2 in silicosis patients has to yet to be determined. There is an interrelationship between TGF- $\beta$ 1 and MMP-9 in the lung. Active MMP-9 will convert latent TGF- $\beta$ 1 to active TGF- $\beta$ 1, which in turn activates pro-MMP-9 released from alveolar macrophage.
- We were able to measure IL-8 in bronchoalveolar lavage fluids (BAL) but levels of other biomarkers are far too low to be measured in the BAL.

In summary, we found that patients with silicosis and sputum atypia had increased risk of lung cancer. Autofluorescent bronchoscopy is an effective method of early detection of lung cancer in patients with silicosis. Patients with silicosis also had evidence of oxidative stress and increased in inflammatory and remodeling biomarkers. The increase in remodeling and inflammatory biomarkers could be related to the fact that they smoke although exposure to silica probably also played a part. While we have measurements of some of these markers in some nonsmokers, we do not have measurements of others. We also need to study a group of health nonsmokers as controls in order to interpret the results better. Further studies are required to sort out the role of silica and smoking in the impairment of lung function in these workers.

The practical values of this study are as follows for patients with silicosis:

- Lung cancer in patients with silicosis can be detected early by autofluorescent bronchoscopy.
- As the changes in the airways of patients in silicosis are due to dust exposure and tobacco smoking, it is vital that patients with silicosis should give up smoking.
- As changes in the airways are related to oxidative stress, it would be helpful for patients with silicosis to have a balanced diet containing antioxidants such as fruits and vegetables in addition to giving up smoking.

As exposure to silica-containing dusts induce silicosis, we recommend the following:

- To employers:

- Reduce silica dust level to the permissible concentration by good ventilation and other appropriate measures and good housekeeping
  - Prohibit smoking in the workplace
- To employees:
  - To use personal protective device when necessary
  - To stop smoking
  - To have a balanced diet containing fruits and vegetables.