Detection of p53 Protein in Induced Sputum After Occupational Exposure to Crystalline Silica

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Learning Objectives

- Describe the properties of the tumor suppressor gene p53 and its protein, and what epidemiological studies have shown about the clinical sequelae of occupational exposure to crystalline silica.
- Compare levels of p53 protein in silica-exposed and non-exposed workers, taking into account the possible effects of smoking.
- Appraise the possible significance of p53 in the pathogenesis of lung cancer and its possible use as a biomarker to identify workers at increased risk.

Abstract

Objective: To examine the possibility of detecting p53 protein in the supernatant of induced sputum (IS) of workers exposed to crystalline silica. Methods: Personal interviews were used to obtain demographic data, occupational and exposure histories, and health habits of the study participants. Sputum samples were collected from all subjects. Results: The all-male study cohort included 35 workers (mean age 43.8 years) exposed to silica and 7 unexposed workers (34.7 years, \( P < 0.05 \)). The mean duration of exposure was 13.4 years, and the range of exposure levels to silica was 0.02 to 0.33 ppm. The mean level of p53 protein was higher in the exposed group compared with the unexposed group (76.47 pg/mL and 62.43 pg/mL, respectively). Conclusions: p53 may serve as a biomarker to identify workers at high risk for developing pulmonary malignancies. IS can detect p53 protein in sputum. (J Occup Environ Med. 2007;49:730–735)

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Lung cancer is one of the leading causes of cancer death in both males and females. The prognosis for patients with non–small-cell lung cancer (NSCLC) is poor, and is related primarily to the stage of disease at the time of diagnosis. There has been little progress in reducing lung cancer mortality by applying conventional screening programs consisting of chest radiograph with or without sputum cytology. Recent advances in molecular biology, however, have provided new approaches that use genetic alterations as potential biomarkers, which can be efficacious for early detection.

Cancer is a multistage process involving the inactivation of tumor suppressor genes and the activation of protooncogenes. Carcinogens can affect any of these stages through genetic and epigenetic mechanisms. In the field of cancer research, p53 is the most prominent in the family of tumor suppressor genes. p53 protein plays an important role in the negative regulation of cell growth, including the regulation of cycles, DNA synthesis and repair, and apoptosis. Mutations in the p53 gene result in a mutant p53 protein with a much longer half-life than that of the wild-type protein, leading to its accumulation in nuclei and in corresponding extracellular fluid. Importantly, they are the most common gene alterations in the development of lung cancer. The frequency of p53 mutations might be as high as 47% in NSCLC and 70% in small-cell lung cancer (SCLC). Muta-
tions in the p53 gene have been found in an increasing proportion of bronchial epithelium derived from normal tissue, through preneoplastic lesions, to malignant tumors.\textsuperscript{12–16}

Crystalline silica was classified in 1997 by the International Agency for Research on Cancer (IARC) as being carcinogenic to humans. One of its pathways involves the generation of oxidants or direct genotoxic activity.\textsuperscript{17} Occupational exposure to silica occurs in a wide variety of occupation settings. Epidemiological studies have conclusively established an association between occupational exposure to crystalline silica and the development of progressive pulmonary inflammation, lung cancer, and fibrosis,\textsuperscript{17} but the mechanisms of this process are not fully understood.

In the present study, we examined p53 protein levels in workers exposed or not exposed to crystalline silica by the methodology of induced sputum (IS), a noninvasive procedure that enables testing extracellular fluid from the respiratory tract.

Materials and Methods

Study Population

The study population consisted of workers from a quarry and a marble factory who had been exposed to silica and workers unexposed to silica in their workplaces. The subjects were referred for evaluation to the National Laboratory Service for Interstitial Lung Disease at the Institute of Pulmonary and Allergic Diseases, Tel-Aviv Sourasky Medical Center. Sputum induction was performed by using a slightly modified method by Pin et al.\textsuperscript{19} Briefly, the subjects were pretreated with β-2 agonists and tested for FEV1, after which they inhaled a hypertonic saline solution (3% NaCl) via an ultrasonic nebulizer (Omron U-22) for about 10 minutes. They were encouraged to cough deeply and expectorate into a sterile cup. Sputum samples were kept at room temperature (for no longer than 2 hours) until processing.

Induced Sputum Processing

The sputum was processed as soon as possible using the method by Popov et al.\textsuperscript{20} with some modifications. Briefly, samples were placed in a petri dish and the plugs were separated by a wooden applicator and transferred to pre-weighted Eppendorf test tubes. After calculating the weight of the plugs, 1:10 Sputum Induction and Processing\textsuperscript{16–18}: forced expiratory volume in 1 second (FEV1, percent predicted) >60%, no history of asthma, chronic lung disease not requiring drug therapy, and no contraindication to receiving Ventolin.

Before comparing the results obtained between the exposed and the unexposed groups, adjustment was made for age, sex, origin, education, and smoking. Each participant completed a questionnaire for the acquisition of demographic data, history of occupations and exposures, medical history, and smoking habits.

Laboratory Methods

We followed the protocol as developed at the National Laboratory Service for Interstitial Lung Disease at the Institute of Pulmonary and Allergic Diseases, Tel-Aviv Sourasky Medical Center. Sputum induction was performed by using a slightly modified method by Pin et al.\textsuperscript{19} Briefly, the subjects were pretreated with β-2 agonists and tested for FEV1, after which they inhaled a hypertonic saline solution (3% NaCl) via an ultrasonic nebulizer (Omron U-22) for about 10 minutes. They were encouraged to cough deeply and expectorate into a sterile cup. Sputum samples were kept at room temperature (for no longer than 2 hours) until processing.

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fraction for determination of crystalline silica content.

**Statistical Analysis**

Comparisons of the differences in the categorical variables between exposed and unexposed subjects were performed by $\chi^2$ or Fisher exact tests and by a non-parametric Mann-Whitney $U$ test (for age). Comparison of p53 levels between the two groups was by the non-parametric Mann-Whitney $U$ test, which is suitable for small samples. Comparison between adjusted means of p53 protein (to smoking and age) was performed by (General Linear Model-General Factorial Analysis procedure) analysis of variance for unbalanced design. This was done on the rank of the p53 variables because of the small number of the workers in the unexposed group. All these tests were two-tailed, and a $P$ value of $\leq 0.05$ was considered statistically significant. The SAS statistical software package version 8.2 (SAS Institute, Cary, NC) was used for all analyses.

**Results**

The all-male study population included 35 workers exposed to silica and 7 workers unexposed to silica. The mean age ($\pm$ standard error) was $43.8 \pm 1.55$ years for the former group and $34.7 \pm 3.26$ for the latter group ($P < 0.05$). The mean duration of exposure to silica was 13.4 (range 0.5–25.0) years. Exposure to silica was monitored at six points of the work area during the work shifts. The range of exposure level was 0.02 to 0.33 mg/m$^3$ (Table 1). Chest x-ray films of the exposed group were normal without pneumoconiosis or other lung disease.

Analysis of the subjects’ smoking habits showed that 15 (42.8%) exposed workers and 1 (14.3%) unexposed worker were non-smokers, 4 (11.4%) and 2 (28.6%), respectively, were past smokers, and 16 (45.7%) and 4 (57.1%), respectively, were current smokers. These differences were not significant. The mean p53 protein level among the smokers (past and current) was almost the same as that of the non-smokers ($74.156 \pm 6.947$ pg/mL, range 37.202–168.866 pg/mL and 78.431 $\pm 15.128$ pg/mL, range 44.504–346.70 pg/mL, respectively). There was no significant difference between smokers and non-smokers with respect to years of exposure to silica ($12.80 \pm 1.18$ years, range 44.504–346.696 pg/mL, respectively) (Table 2).

Finally, the mean p53 protein level was higher for the exposed subjects ($76.47 \pm 8.70$ pg/mL, range 37.20–346.70 pg/mL) compared with for the unexposed subjects ($64.43 \pm 9.42$ pg/mL, range 7.16–79.86 pg/mL). This difference did not reach a level of statistical significance. These results did not change significantly after adjusting for age and smoking: mean level of p53 protein was still higher for the exposed subjects, 75.67 $\pm$ 8.49, compared with for the unexposed subjects, 66.32 $\pm$ 19.83.

**Discussion**

The current study applied IS technology in an attempt to demonstrate an association between exposure to silica and high levels of p53 protein in exposed subjects’ sputum. The results showed that IS was an effective, noninvasive, and feasible technique for detecting p53 protein accumulation in the extrapulmonary fluid of asymptomatic silica-exposed individuals.

In humans, a large fraction of inhaled crystalline silica persists in the lungs and can cause progressive pulmonary inflammation, lung cancer, and fibrosis. Based on the results of an in vitro study that indicated that quartz particles bind to DNA by hydrogen bonding, on another study that showed that the DNA damage induced by crystalline silica is mediated by the formation of oxygen radicals on the silica surface, and on the finding of small quartz particles in the nuclei and mitotic spindles of alveolar epithelial cells exposed in culture, Daniel et al. proposed a model for silica carcinogenesis. They considered that the binding of crystalline silica to cellular DNA may produce DNA damage in vivo by anchoring DNA within a few Angstroms of the sites of oxygen-free radical production on the silica surface. This anchoring enables short-lived toxic radicals, such as the hydroxyl radicals, to reach DNA bases and induce the DNA

### Table 1

**Baseline Characteristics of the Study Population**

<table>
<thead>
<tr>
<th>Sex*</th>
<th>Unexposed ($N = 7$)</th>
<th>Exposed ($N = 35$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>7</td>
<td>35</td>
</tr>
<tr>
<td>%</td>
<td>100.0</td>
<td>100.0</td>
</tr>
<tr>
<td>Education (yr)*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1–12</td>
<td>4</td>
<td>8</td>
</tr>
<tr>
<td>%</td>
<td>57.1</td>
<td>22.9</td>
</tr>
<tr>
<td>&gt;12</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>%</td>
<td>42.9</td>
<td></td>
</tr>
<tr>
<td>Origin*</td>
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<td></td>
</tr>
<tr>
<td>Asia/Africa</td>
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<td>20</td>
</tr>
<tr>
<td>%</td>
<td>42.9</td>
<td>57.1</td>
</tr>
<tr>
<td>Europe/Americ</td>
<td>2</td>
<td>11</td>
</tr>
<tr>
<td>%</td>
<td>28.6</td>
<td>31.4</td>
</tr>
<tr>
<td>Israel</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td>%</td>
<td>28.6</td>
<td>11.4</td>
</tr>
<tr>
<td>Smoking*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>1</td>
<td>15</td>
</tr>
<tr>
<td>%</td>
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<td>42.8</td>
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<tr>
<td>Yes</td>
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<td>16</td>
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<tr>
<td>%</td>
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<td>45.7</td>
</tr>
<tr>
<td>Past</td>
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<tr>
<td>%</td>
<td>28.6</td>
<td>11.4</td>
</tr>
<tr>
<td>Age*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean (+standard error)</td>
<td>37 (3.26)</td>
<td>43.8 (1.55)</td>
</tr>
</tbody>
</table>

*Non significant.
damage critical for mutagenesis, neoplastic cell transformation, and carcinogenesis. The binding of crystalline silica to DNA may also lead to DNA damage by interfering with the replication, repair, or expression of DNA or by altering the mitotic process.

The evidence that quartz-induced neoplastic transformation and chromosomal aberrations in cells in culture and the finding of increased levels of sister chromatid exchange and chromosomal aberrations in peripheral blood lymphocytes in subjects exposed to dust containing crystalline silica further support the hypothesis that crystalline silica is capable of producing DNA damage in living cells.

The p53 tumor suppressor protein is a transcription factor that regulates the transcription rate of several genes involved in the regulation of the cell cycle, DNA, and apoptosis. Under normal circumstances, cells respond to external damage stimuli by activating the regulation of exspond to external damage stimuli der normal circumstances, cells respond to external damage stimuli by activating the regulation of exspond to external damage stimuli. Indeed, apoptosis of individual cells may present a protective mechanism against neoplastic development in the organism by eliminating damaged cells. Normal p53 function was shown to be crucial in the induction of apoptosis in human and marine cells following DNA damage. Thus, p53-mediated apoptosis is an important part of the tumor suppressor phenotype and so p53 deficiency may permit a population of genetically damaged cells to escape the normal process of apoptotic deletion.

Several genotoxic agents, including ionizing radiation, ultraviolet radiation, and certain chemicals, have been reported to cause p53 upregulation and marked decreases in apoptosis after exposure to radiation were found to be correlated with the occurrence of p53 mutations in some transgenic mice. Wang et al. hypothesized that p53 may play a key role in silica-induced apoptosis and that abnormal regulation of p53 by silica may contribute to the development of lung cancer as well as silicosis. To test this hypothesis, they carried out in vitro and in vivo studies and found that silica induces p53 transactivation via induction of p53 protein expression and phosphorylation of p53 protein at serine 392, and that p53 plays a crucial role in the signal transduction pathways of silica-induced apoptosis. This finding may provide an important link in understanding the molecular mechanisms of silica-induced carcinogenesis in the lung.

The phosphorylation of serine 392 can stabilize the tetramer formation of tumor suppressor protein p53, resulting in an increase of the half-life of p53 protein, in elevated p53 transcription activity, and in the accumulation of p53 protein in the cells.

One of the most common somatic genetic alterations in human cancers involves the loss of the inhibitory function of the p53 tumor suppressor gene products. In many cases, a common mechanism is the loss of one normal p53 allele or the point mutation of another p53 allele, or both. The resultant effect is a paradoxical increase in intracellular levels of the p53 protein. Because many mutant forms of p53 have a much longer half-life than the wild-type p53, the mutant form of p53 can bind to the wild-type, stabilize its normally rapid degradation and extend its half-life. The intracellular accumulation of mutant p53 protein in cells in culture results in a corresponding increase in p53 protein in the extracellular supernatant. Similarly, individuals with tumors that have increased levels of mutant p53 protein can also have high levels of p53 in extracellular fluids, such as serum. Thus, the detection of protein implies the presence of mutation.

Immunohistochemical studies of the aerodigestive tract detected p53 proteins in high percentages in pre-malignant regions of dysplasia and in situ carcinoma, but no overexpression was seen in normal bronchial mucosa. It is noteworthy that p53 mutations were found in regions adjacent to primary tumors and in distant locations in the aerodigestive tract, supporting the cancerization concept. This finding suggests that genetic damage resulting in p53 accumulation and possibly that p53 gene mutation may be an early event in lung carcinogenesis, preceding lung cancer invasion, and that the mutant p53 protein may be a good target marker for early cancer detection.

Luo et al. reported that the p53 protein can be detected in serum in

<table>
<thead>
<tr>
<th>TABLE 2</th>
<th>p53 pg/mL in Relation to Exposure</th>
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<tbody>
<tr>
<td></td>
<td>Mean*</td>
</tr>
<tr>
<td>Exposed (N = 35)</td>
<td>76.47 ± 8.7</td>
</tr>
<tr>
<td>Unexposed (N = 7)</td>
<td>62.34 ± 9.42</td>
</tr>
</tbody>
</table>

*Non significant.
some lung cancer cases with p53 alteration in the tumor tissue. Both K-ras and p53 mutations have been identified in sputum samples from patients who were diagnosed as having adenocarcinoma of the lung up to 1 year later.50 Hustafvel-Pursiainen et al.51 observed p53 and K-ras mutations in lung cancers from smokers who had not smoked for decades. Bennett et al.46 reported that p53 accumulation was detected in atypical cells with a higher percentage in severe dysplasias than in mild dysplasias of the bronchial epithelia.

The detection of p53 protein accumulation in preneoplastic cells in sputum50,52–54 and the detection of p53 mutation prior to the diagnosis of lung cancer15 suggest that p53 mutations can be early events in the development of lung cancer and that p53 accumulation reflects tissue alterations in p53 at the gene and/or protein level, and thus may be an early biomarker for assessing lung cancer risk.

In an era in which both computed tomographic imaging and fluorescence bronchoscopic hold promise for early detection of lung pathology55 and in which new chemoprevention agents are being developed,56 a noninvasive screening technique to detect biomarkers that represent an early stage in lung carcinogenesis would be especially valuable in order to identify individuals at high risk to develop malignant disease. The IS technique is already widely used for other diagnostic purposes in medicine.57 We have shown that it was capable of detecting elevated levels of p53 protein in the sputum of asymptomatic workers exposed to silica. These results support the hypotheses that p53 plays an important role in the carcinogenesis process of silica in the lung, that p53 may serve as biomarker to identify workers at high risk, and that IS technology is suitable for the purposes of screening and monitoring in this setting.

Acknowledgment

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References

13. Weithege T, Voss B, Muller KM. p53 accumulation and proliferating–cell nu-


