

Measurement of Lung Cancer Tumor Markers in a Glass Wool Company Workers Exposed to Respirable Synthetic Vitreous Fiber and Dust

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Abstract

Background: Occupational exposures to respirable synthetic vitreous fiber (SVF) and dust are associated with many lung diseases including lung cancer. Low-dose computed tomography is used for screening patients who are highly suspicious of having lung carcinoma. However, it seems not to be cost-effective. Serum biomarkers could be a useful tool for the surveillance of occupational exposure, by providing the possibility of diagnosing lung cancer in its early stages.

Objective: To determine if serum carcinoembryonic antigen (CEA) and cytokeratin fragment (CYFRA) 21-1 levels in workers exposed more than normal population to respirable SVF and dust may be used as indicators of progression towards lung cancer.

Methods: An analytic cross-sectional study, including 145 personnel of a glass wool company, along with 25 age-matched healthy individuals, was conducted to investigate the relationship between occupational exposure to respirable SVFs and dust and serum levels of two lung/pleura serum tumor markers, CEA and CYFRA 21-1, measured by ELISA.

Results: Individuals exposed to higher than the recommended levels of respirable SVF had higher serum concentrations of CEA and CYFRA 21-1, compared to controls ($p=0.008$ and 0.040 , respectively), as well as in comparison to those exposed to lower than recommended OSHA levels ($p=0.046$ and 0.033 , respectively). Workers with >9 years work experience, had significantly ($p=0.045$) higher levels of serum CYFRA 21-1 than those with ≤ 9 years of experience.

Conclusion: It seems that working for >9 years in sites with detectable levels of respirable SVF and dust would increase the levels of known lung cancer serum tumor markers. Transferring these workers to sites with respirable SVF concentrations lower than the limit of detection in the air is recommended.

Keywords: CYFRA 21-1; Carcinoembryonic antigen; lung neoplasms; Occupational health; Synthetic vitreous fiber; Dust

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Introduction

Repeated long-term exposure to certain irritants in work place is associated with an array of lung diseases that might progress even after the exposure ceases. Work-related respiratory diseases constitute approximately 70% of all occupational disease mortalities.¹ The main categories of these diseases are lung cancer, chronic obstructive pulmonary disease (COPD), occupational asthma, pneumoconiosis, and other non-cancerous respiratory diseases such as diffuse pleural thickening and pleural plaques.² Certain employees, including glass wool factory workers, are at a higher risk of developing occupational lung disease owing to the nature of their work setting, and environment.

Glass wool is made from local raw materials like sand, and contains up to 80% recycled glass, which makes it primarily silica-based; it also contains various amounts of other oxides (*eg*, aluminum, boron, calcium, or iron oxides). Therefore, synthetic vitreous fibers (SVF) are in fact not a single chemical entity; they are a group of amorphous polysilicates.³

The level of exposures to respirable SVF and dust can be assessed from the breathing zone air samples that are representative of the 8-hour work day.⁴ According to Occupational Safety and Health Administration (OSHA) recommended occupational exposure limits issued by the American Conference of Governmental Industrial Hygienists (ACGIH), the exposure limit for SVF is 1 fiber/mL 8-hour Total Weighted Average (8-hr TWA).⁴

SVF is classified as Group 3 (not classifiable as carcinogen to humans) by the International Agency for Research on Cancer (IARC) and its reclassification from Group 3 to Group 2B (possibly carcinogenic to humans) has remained controversial.^{4,5} There are studies demonstrating occu-

pational exposures to respirable SVF and dust are associated with the development of pneumoconiosis (particularly silicosis), lung cancer, pleural mesothelioma, pulmonary tuberculosis, and airways diseases.^{6,7}

Lung cancer can be conceptualized as the joint consequences of synergistic interactions among risk factors such as genetic predisposition, the effect of cigarette smoking, respirable SVF and dust exposure, among other factors.⁸⁻¹⁰ The early detection of lung cancer dramatically reduces the disease mortality. The overall 5-year survival rate for lung cancer is 17%. However, if it is diagnosed at stage Ia, this rate increases to 80%.¹¹ As a result, a non-invasive test for early diagnosis of lung cancer would thus improve the patients' survival. Serum biomarkers reflecting specific tumor tissue remodeling processes may be valuable diagnostic tools for lung cancer. Carcinoembryonic antigen (CEA) and cytokeratin fragment (CYFRA) 21-1, the two serum biomarkers for lung cancer, have been studied extensively.¹²⁻¹⁷

CYFRA 21-1 is a fragment of cytokeratin 19. It is expressed in the unstratified and pseudostratified epithelium lining of the bronchial tree;¹⁵ its expression is well-maintained even during the process of transformation of bronchial epithelium to malignant cells.¹⁸ Many studies demonstrated that high expression of CYFRA 21-1 in non-small cell lung cancers (NSCLC) has diagnostic and prognostic value. CYFRA 21-1 has been demonstrated to be a sensitive biomarker for NSCLC.^{16,19-22} CEA is a glycoprotein involved in cell adhesion and already recognized and used as a tumor marker in some malignancies including colorectal cancer.²³ Additionally, serum CEA levels seem to carry prognostic value in lung cancer patients.²³

We conducted this study to determine if serum CEA and CYFRA 21-1 levels in workers exposed to respirable SVF and dust may be used as indicators for progres-

sion towards lung cancer.

Materials and Methods

A single-center analytic cross-sectional study, including 150 workers and employees of a glass wool company, Shiraz, Iran, along with 25 age-matched non-smoker healthy individuals, was carried out to investigate the relationship between occupational exposure to respirable SVF/dust and serum levels of two lung cancer serum markers, CEA and CYFRA 21-1. To eliminate the confounding factors as much as possible only workers/employees working 8 hours a day were included in this study. In addition, we excluded those workers/employees with a medical history of dermatologic disorders such as erythema multiforme, liver diseases such as cirrhosis, renal problems such as chronic kidney disease, pulmonary problems such as tuberculosis and asthma, and history or presence of any malignancies.

Of the 150 workers, 145 met the inclusion criteria. Their serum samples were collected for the measurement of CEA and CYFRA 21-1. Regarding non-occupational settings, indoor air concentrations of SVF are very low unless there is a disturbance in the fiberglass insulation system or ceiling boards of the building. We therefore selected our control group from healthy individuals not exposed to SVF, to further eliminate confounding factors.

Air Sampling and Analysis

Exposure assessments of respirable SVF/dust were conducted for three consecutive days at six sites of the factory—furnace, pre-insulation, palletization and packaging, storehouse, repairs and maintenance, and administrative division. Phase contrast microscopy (NIOSH Method 7400a)²⁴ was used to measure the levels of SVF.

Blood Samples and ELISA

Five ml of venous blood was collected from each participant. The samples were centrifuged at 1200×g for 10 min at 4 °C within 1 hour of collection. The serum was transferred to a fresh tube and stored at -80 °C until analysis. Levels of CEA and CYFRA 21-1 in the sera of participants were measured by a quantitative enzyme-linked immunosorbent assay (ELISA) kit (CYFRA 21-1 kit: Immuno-Biological Laboratories, Inc, USA; CEA kit: Fujirebio Diagnostics AB, Sweden) according to the manufacturer.

Subgroup Definitions

Pulmonary effects of exposure to respirable SVF/dust are determined by the “3 D’s,” dose, dimension, and durability.²⁵ The number of years each person had been worked at the site of sampling was used as an estimation of exposure duration. To further categorize our data, we considered the median (9 years), and 25th and 75th percentiles (5 and 13 years respectively) of work experience cut-off points for time of exposure. As a rough calculation of cumulative exposure to respirable SVF and dust, we used the multiplicand of exposure years to measure 8-hr TWA.

Ethics

The study was approved by the Ethics Committee of the Shiraz University of Medical Sciences. The protocol was in accord with the declaration of Helsinki.²⁶ An informed consent was taken from each participant. Those participants who did not agree to participate in the study were also excluded from the study.

Statistical Analysis

SPSS® for Windows® ver 16 (SPSS Inc, Chicago, IL, USA) was used for data analysis. Shapiro-Wilk test was used to determine if studied variables were normally

Table 1: Measured levels of respirable synthetic vitreous fiber (SVF) and dust with phase contrast microscopy (NIOSH Method 7400a) in different sites of the studied glass wool company

Site	SVF (fiber/mL)	Respirable dust (mg/m ³)
Furnace	0.610	3.3
Pre-insulation	1.680	4.4
Palletization and packaging	3.400	8.9
Storehouse	0.580	6.6
Repairs and maintenance	0.007	2.2
Administrative division	<LOD*	<LOD
OSHA TWA permissible exposure limit†	1.000	3.0

*Limit of detection; †Occupational Safety and Health 8-hour time-weighted average permissible exposure limit

distributed. Variables with normal distribution are presented as mean (SD); otherwise, as median. Frequencies are presented as percentages. Mann-Whitney U and Kruskal-Wallis tests were used to analyze the difference among groups. Pearson correlation coefficient was used to assess correlate between two variables. A p value <0.05 was considered statistically significant.

Results

Serum CEA and CYFRA 21-1 Levels

The mean age of studied workers was 37.5 (SD 6.3) years; the mean age of the comparison group participants was 37.5 (SD 9.5) years (p=0.98). The 8-hour TWA of respirable SVF and dust, along with OSHA recommended levels are presented in Table 1. No significant difference was observed in serum CEA and CYFRA 21-1 levels between the exposed and non-exposed participants (p=0.106 and 0.360, respectively). Regardless of exposure duration, those exposed to SVF >1 fiber/mL, had a higher mean serum CYFRA

21-1 level than non-exposed comparison group participants (Fig 1A; 0.315 vs 0.243 ng/mL; p=0.040). The same was true for serum CEA level, (Fig 1B; 1.742 vs 1.140 µg/L; p=0.008). The mean serum CYFRA 21-1 level was also significantly (p=0.031) higher in those exposed to respirable dust >3 mg/m³ than the comparison group (Fig 1C; 0.306 vs 0.238 ng/mL). So did the mean CEA level (Fig 1D; 1.628 vs 1.140 µg/L; p=0.019). However, no significant difference was observed when the mean serum CEA and CYFRA 21-1 levels were compared between the comparison group and those exposed to lesser than 8-hr TWA OSHA recommended respirable SVF and dust concentrations (p=0.997 and 0.902, respectively).

The mean serum CYFRA 21-1 level was significantly (p=0.033) higher in workers exposed to SVF ≥1 fiber/mL than in those exposed to <1 fiber/mL (Fig 2A; 0.315 vs 0.230 ng/mL; p=0.033). The same was true for the mean serum CEA level (Fig 2B; 1.742 vs 1.377 µg/L; p=0.046). No significant difference was observed in the mean serum CYFRA 21-1 and CEA levels between workers exposed to respirable dust ≥3 mg/m³ and those exposed to <3 mg/m³.

We subdivided workers according to the median of their work experience into two groups. Working >9 years was associated with a higher mean serum CYFRA 21-1 level (Fig 2C; 0.315 vs 0.229 ng/mL; p=0.045). We then excluded workers exposed to SVF levels below the limit of detection (<LOD) and found that working >9 years in sites exposed to SVF >LOD, was associated with a higher mean serum CYFRA 21-1 level (Fig 2D; 0.358 vs 0.229 ng/mL; p=0.028). No such association was observed in those exposed to SVF <LOD. No significant association was also observed between SVF/dust cumulative exposures and serum CYFRA 21-1 and CEA levels.

When comparing smoker and non-

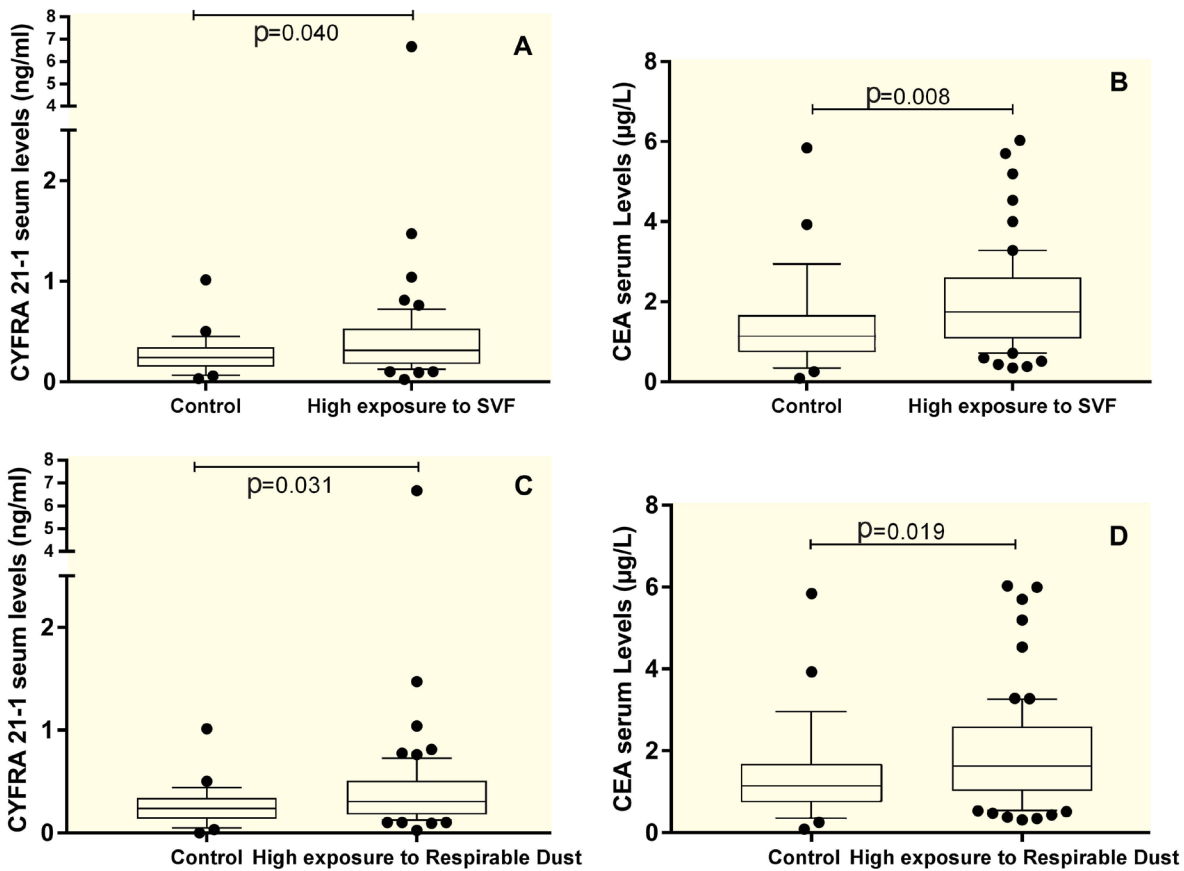


Figure 1: Box-and-whisker diagram. (A) Cases exposed to SVF >1 fiber/mL (high exposure) had higher levels of serum CYFRA 21-1 than the comparison group. (B) In those with high exposure to SVF, higher levels of serum CEA was observed than in the comparison group. (C) In those exposed to respirable dust >3 mg/m³ (high exposure), serum CYFRA 21-1 levels were higher than the comparison group. (D) In those with high exposure to respirable dust, higher levels of CEA was recorded in comparison to the comparison group.

smoker workers/employees, There were significantly higher levels of CYFRA 21-1 and CEA in the sera of smokers (0.392 vs 0.279 ng/mL; p=0.027 and 3.491 vs 1.319 µg/L; p<0.0001, respectively). Since the frequency of smokers was low in the exposed group (n=13), we excluded them from our data, and repeated the statistical analyses. Serum CYFRA 21-1 and CEA levels were still higher in those exposed to SVF ≥1 fiber/mL; the results were however not significant (p=0.056 and p=0.062; respectively). The mean serum CYFRA 21-1 level was significantly higher in those with

wok experience >9 years (median) compared the others (0.306 vs 0.230 ng/mL; p=0.039); it was not true for the mean CEA level.

Discussion

We found that regardless of duration of exposure, individuals exposed to levels higher than the OSHA recommended values of respirable SVF and dust, had significantly higher serum CYFRA 21-1 and CEA levels, in comparison to a comparison group and also compared to those exposed to levels

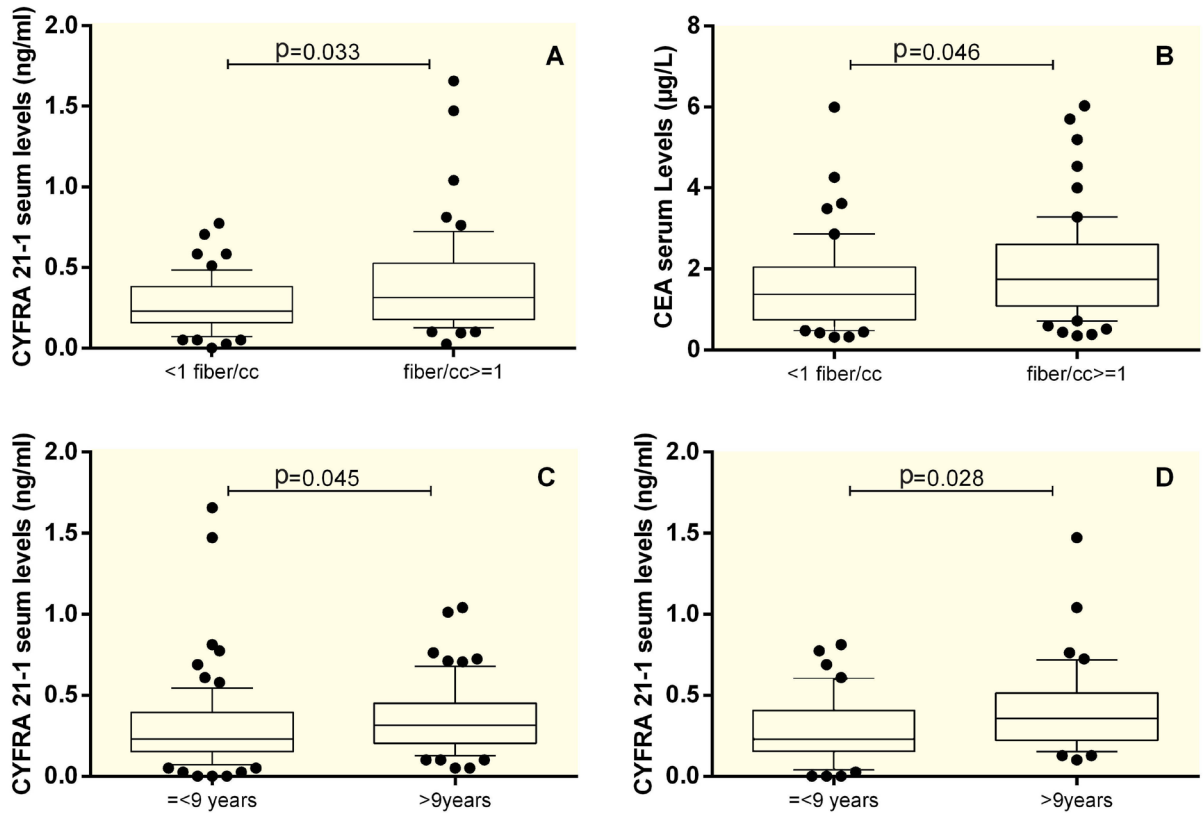


Figure 2: Box-and-whisker diagram. (A) Serum CYFRA 21-1 and (B) CEA levels were significantly higher in workers exposed to ≥ 1 fiber/mL SVF. (C) Working >9 years was associated with higher CYFRA 21-1 serum levels. (D) When excluding those exposed to SVF levels below the limit of detection (LOD), working >9 years in sites exposed to SVF $> LOD$, was associated with higher serum CYFRA 21-1 levels.

lower than the OSHA recommended values. Furthermore, workers with >9 years of work experience, had a higher mean serum CYFRA 21-1 level compared to those with ≤ 9 years of work experience. We also observed significantly higher levels of CYFRA 21-1 and CEA in the sera of smokers. Excluding smokers, the mean serum CYFRA 21-1 level was found significantly higher in workers with work experience >9 years than those without.

Nowadays, low-dose computed tomography is used for screening patients highly suspicious of having lung carcinoma.^{27,28} However, the cost-effectiveness of this screening technique for lung cancer is still under debate.²⁹ Serum biomarkers could

be a practical and useful tool for the surveillance of occupational exposure and workers' health, by providing the possibility of diagnosing pulmonary complications in early stages, and predicting their prognosis.

Cytoskeleton 19 (CK19) is normally expressed in bronchial epithelial cells, and type I and type II alveolar pneumocytes.³⁰ After transformation of these cells to neoplastic cells, during apoptosis, caspase 3 cleaves CK19 with subsequent release of its soluble fragment, CYFRA 21-1 into the serum,³¹ which found as a marker for poor prognosis in NSCLC patients.^{14,15} There are studies demonstrating that chronic airway inflammatory diseases, chest trauma,

and acute respiratory distress syndrome (ARDS) are associated with increased CYFRA 21-1 levels in the broncho-alveolar lavage (BAL) fluid^{30,32} and that serum CYFRA21-1 might be a predictor of progression towards ARDS in patients with severe chest trauma.³⁰ In our study, we observed higher serum levels of CYFRA 21-1 in those exposed to higher amounts of SVF/dust, smokers, and those with longer duration of exposure. It can be assumed that lung tissue injury caused by respirable SVF/dust and tobacco smoke,³³ resulted in the increased CYFRA21-1 levels in the sera of these individuals. None of these levels however exceeded the cut-off level of 15 ng/mL previously suggested by Kim, *et al*,³⁴ for the diagnosis of lung cancer. Whether these cases will progress to lung cancer³⁵ should be determined in future follow-ups.

Along with measurement of serum CYFRA 21-1, serum CEA levels have been shown to be of prognostic value in NSCLC.^{17,23,36} In this study, we observed increased levels of CEA in the sera of workers with higher levels of exposure to respirable SVF/dust in comparison to other workers and also the comparison group. No correlation was found between serum CYFRA 21-1 and CEA levels. For serum CEA level to be diagnostic, a combination with other lung cancer markers such as squamous cell carcinoma (SCC) antigen, neuron-specific enolase (NSE), and CYFRA 21-1 should also be used.^{17,36} The higher levels of CEA in these workers could be due to lung tissue injury. Lin and colleagues showed increased expression of a member of CEA gene family after hyperoxic exposure and lipopolysaccharide (LPS) instillation in their mouse model, which appeared to be a marker of alveolar epithelial cell replacement after lung injury.³⁷

Our results indicated that working >9 years in sites with detectable levels of respirable SVF may result in increased serum levels of known tumor markers for

TAKE-HOME MESSAGE

- Regardless of the exposure duration, those exposed to levels of respirable SVF and dust higher than the OSHA recommended values, had significantly higher serum CYFRA 21-1 and CEA concentrations than a comparison group.
- Workers with more than 9 years exposure to SVF, had higher levels of CYFRA 21-1 in their sera compared to those with ≤9 years of exposure.
- Significantly higher serum CYFRA 21-1 and CEA levels were observed in smokers than non-smokers.

lung cancer. Therefore, transfer of these workers to non-exposed sites with respirable SVF level <LOD in the air is highly recommended.

In this study, we confronted several limitations that could not be eliminated. First of all, phase contrast microscopy (PCM) used to measure SVF levels, cannot detect fibers with diameters <0.25 μm. Second, in addition to being exposed to respirable SVF and dust, the participants were also exposed to several other carcinogens like formaldehyde, welding fumes, *etc* in their work place that can affect the measured markers levels. Finally, the statistical significance of our results may not be robust enough to yield distinct information for the occupational health/clinical use. In future studies, as well as follow-up of these participants for progression towards any lung disease, considering other paraclinical data, like chest x-ray or CT scan, spirometry, and diffusing capacity of the lungs for carbon monoxide (DLCO), would help further clarify the value of these markers for screening of lung cancer or other respiratory diseases in these workers.

Conflict of Interests: None declared.

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