Serum Malondialdehyde and Urinary Neopterin Levels in Glass Sandblasters Exposed to Crystalline Silica Aerosols

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ABSTRACT
The aim of this cross-sectional study was to explore the association of crystalline silica aerosols exposure with malondialdehyde in blood serum and urinary neopterin and explore their potential as biomarkers of their external exposure. Nonsmoking and healthy male glass sandblasters and control population were randomly selected for this study. All groups were monitored for their personal exposure to crystalline silica according to NOISH method No. 7601. High Performance Liquid Chromatography (HPLC) was used to for analysis of malondialdehyde of blood serum and urinary neopterin, and creatinine in all study participants. The mean of personal exposure to crystalline silica aerosols in glass sandblasters was 164 µg/m³ (SD: 112) compared with less than 0.006 mg/m³ for control group that was even below detection limit. The mean of blood serum malondialdehyde of sandblasters (49.08±19.05 µmole/l) was significantly higher than that of control population 1.92±0.33 µmole/l (p<0.001). Urinary neopterin of sandblasters was 10.85±3.61 mmole/mole creatinine which was also significantly higher than control group 4.71±1.88 mmole/mole creatinine (p<0.001). Correlation between occupational exposures of glass sandblasters to crystalline silica with blood serum malondialdehyde was significant (r²=0.279, p<0.01). Malondialdehyde of blood serum and urinary neopterin could be regarded as biomarkers of exposure to crystalline silica aerosols.

Keywords: Glass Sandblaster, Crystalline silica, Biomonitoring, Malondialdehyde, Neopterin

INTRODUCTION
Crystalline silica (SiO₂) is the second most abundant compound on the earth crust [1] and many occupational groups working at various sites such as roads, agricultural, mining, power plants, quarries, construction, metal and glass industries are exposed to crystalline silica aerosols [2-4]. Cytotoxicity of crystalline silica in form of silicosis has been known from ancient Greek times [5]. In recent years exposure to crystalline silica aerosols are recognized as the cause of fibrogenic reactions and confirmed human carcinogen as well [6-7]. Despite of rigorous efforts suggested by International Labour Organization for
controlling worker’s exposure to crystalline silica, it still afflicts millions of workers engaged in hazardous dusty occupations in many countries and silicosis continues to be one of the most important occupational health problems in the world [8].

Progression of silicosis in occupational groups in most cases takes lifetime to manifest itself in chest radiological or spirometric examinations [9]. Silica particles are important occupational hazards in a wide variety occupations and exposure may result in DNA damage and lipid peroxidation through oxidative stress [10]. Hence, possible biomonitoring of early effects of worker’s exposure may display a more explicit picture of the effects of exposure to crystalline silica aerosols and it would be useful to prevent the progression of disease [11].

In a few very recent studies, malondialdehyde in blood serum an indicator of lipid peroxidation [12-13] and urinary neopterin, another indicator of oxidative stress resulting from cell-mediated immune activation are introduced as potential biomarkers of exposure to respirable crystalline silica dusts [14-15].

The aim of this cross sectional study was to investigate serum malondialdehyde and urinary neopterin as potential biomarkers in a group of young healthy glass sandblasters who were exclusively exposed to silica dusts.

### MATERIALS AND METHODS

Twenty five glass sandblasters along with another twenty five control workers working in restaurants and warehouses without active exposure to silica dusts from Varamin region in southern part of Tehran were selected for this study. All workers in this study were selected from nonsmoking working population and were not under any special medications. A variety of analytical techniques are offered for the quantitative determination of crystalline silica [16]. In this study, visible spectroscopy according to the NIOSH method No. 7601 [17] was applied and airborne respirable dust samples were collected from breathing zone of the study population on mixed cellulose filters (25 mm diameter, 0.8 mm pore size), by using a cyclone separator (Higgins and Dewell) at the flow rate of 2.2 l/min for a maximum volume of 800 liters. Standards of crystalline silica with known purity were prepared by spiking 20, 40, 80, 160, 320 μg of quartz (84% purity) on mixed cellulose filters. Filter samples, standards and blanks (mixed cellulose filter) were digested with HNO₃ and subsequently washed with H₃PO₄ and suspended crystalline silica were filtered and dissolved with HF and molybdate reagent was added and subsequently absorbance of silicomolybdate complex was read at 820 nm by a Cecil Spectrophotometer Model 2021.

Blood and urine samples from glass sandblasters and control groups were taken after their consent by paramedical personnel in early mornings and kept from direct light. Serum was recovered after centrifugation at 3,000 rpm for 15 min and subsequently serum and urine samples were stored at temperature of -20°C for analysis within two weeks.

Urinary neopterin was measured by a modified high-performance liquid chromatography (HPLC) method using a UV-Visible detector according to a modified method by Castro et al. [18]. In this method, urine samples were centrifuged for 10 minutes at 5000 RPM and then 10 μl of supernatant was added to 990μl of mobile phase (50% methanol and 50% 30mM potassium dihydrogen phosphate). Standard solutions were prepared by neopterin purchased from Sigma Co. in concentration range of 100-1000ng/ml. Samples and standards were injected to Merck Hitachi model L-7420 HPLC-UV equipped with RP18 column and absorbance was measured at the wavelength of 353 nm. And urinary creatinine analysis was conducted according to method developed by Azari et al. [19] urine samples were cleaned up by acidification and double centrifugation at 6000rpm and diluting of samples 100 times by mobile phase (10% acetonitrile and 90% ammonium hydrogen diphosphate10mM). Standard solutions of creatinine purchased from Sigma Co. were prepared in concentration of 100-500μMolar and 20 μl of samples and standards were injected to HPLC equipped with a RP18 column and ultraviolet detector (Merck Hitachi model L-7420) at the wavelength of 235 nm. The neopterin levels are reported as micromoles of neopterin per mole of creatinine.

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<th>Table 1. Demographical data of nonsmoker glass sandblasters and control</th>
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<td>Demographic data</td>
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<td>Age (years)</td>
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<th>Table 2. Typical exposure to SiO₂ aerosols and biological effective dose of external dose</th>
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<td>SiO₂ external dose and biological effective dose</td>
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<tr>
<td>SiO₂ (mg/m³)</td>
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<td>Serum malondialdehyde (µmole/L)</td>
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<td>Urinary neopterin (µmole/mole Creatinine)</td>
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*less than detection limit 0.006mg/m³
Blood serum of the glass sandblasters and control groups was analyzed for malondialdehyde (MDA) according to method developed by Karatas et al. [20]. Blood serum samples were centrifuged for 10 minutes at 5000 RPM and 25 µl of serum were mixed with 250 µl of 0.1 M perchloric acid and finally 700 µl of double distilled water were added centrifuged for 10 minutes. And, finally 20 µl of prepared samples were injected to Merck Hitachi model L-7420 HPLC-UV equipped with RP18 column with mobile phase of 65% methanol and 35% potassium dihydrogen phosphate with flow of 1.5 ml/min. Standard solutions of malondialdehyde (MDA) were made by 1,1,3,3-tetra-hydroxypropane from Sigma Co. with 0.1 M perchloric acid in range of 1-100 µmole/L.

All data were analyzed with SPSS 15 software for statistical analysis, with assumption of the normality of the data, independent t-test was used to compare the mean between two groups and Pearson correlation coefficient was used to measure relation between exposure to crystalline silica with serum MDA and urine Neopterin. p-values less than 0.05 were taken as statistically significant.

RESULTS

All workers in this study were nonsmokers and regarding to demographic parameters such as age, work history and working durations, there was no statistically significant difference in the mean age and daily working hours and years of work between glass sandblasters and control group (Table 1). The geometric mean of personal exposure to respirable crystalline silica ($SiO_2$) dusts in sandblaster was 164 (±112 µg/m$^3$) whereas the mean of exposure to crystalline silica was below detection limit (0.006 mg/m$^3$) in control group (Table 2).

Mean serum MDA of glass sandblasters 49.08±19.05 µmole/L versus control group 1.92±0.33 µmole/L was found to be significantly higher ($p<0.001$). There was statistically significant correlation between crystalline silica exposure and serum MDA ($p<0.01$).

Mean urinary Neopterin of glass sandblasters 10.85±3.61 μmol/mol creatinine versus mean control group 4.71±1.88 μmol/mol creatinine was found to be significantly higher ($p<0.001$) (Table 2). However, there was no statistically significant correlation between crystalline silica exposure and neopterin ($p>0.05$).

DISCUSSION

Concern for workers’ exposure to crystalline silica as causative agent for onset of silicosis and lung cancer has been an internationally recognized [21]. International Labor Organization (ILO) has recommended further research in regards to silicosis and preventive measures [22]. In this study, a special group of workers (glass sandblasters) were studied due to problems reported by the Ministry of Health experts. Glass sandblasters (nonsmokers) are unique in regards to their exclusive exposure to crystalline silica aerosols. Exposure of sand blasters in this study was comparable with a previous study [23] and their exposure in term of geometric mean (0.164 mg/m$^3$) was much higher than the geometric mean of their counterparts in the United Kingdom with an exposure level of 0.09 mg/m$^3$ [24] and threshold limit value (TLV) set by ACGIH at 0.025 mg/m$^3$ [25].

Acute and chronic exposure to crystalline silica aerosols has been reported to trigger lipid peroxidation processes through repeated phagocytosis of crystalline silica particles and the enhanced generation of Reactive Oxygen Species [26]. And, in various in vitro [27-30] and in vivo animal studies [31], exposure to crystalline silica particles has been associated with increased level of malondialdehyde as biomarker of lipid peroxidation. Orman et al. investigated the lipid peroxidation of workers exposed to cement dust containing crystalline silica through measuring serum malondialdehyde (MDA) and erythrocyte glutathione levels in workers and concluded that serum MDA could be accepted as an indicator of oxidative injury in workers [13]. Our study confirms Orman et al. findings about plasma MDA a biomarker of crystalline silica exposure and also shows a significant correlation between exposures with levels of crystalline silica exposure and serum malondialdehyde of glass sandblasters. Considering another study stating significantly higher concentration of serum MDA in silicotic patients than control population [32], increased serum MDA could have a potential of being an early effect prior to development of silicosis, if exposures persist at unaccepted risk level.

Recently few studies suggested that exposure to crystalline silica aerosols could effect immunological functions T lymphocytes, neutrophils and immunoglobulins [14-15]. Altindag et al. recently have reported urinary or serum neopterin as biological indicator of early effect as a result of crystalline silica exposure in foundry workers, and have linked excess neopterin a product as result of oxidative stress [15]. Our results for urinary neopterin support Altindag et al. findings by demonstrating statistically significant higher concentrations in glass sandblasters than control population. However, significant correlation of urinary neopterin with various magnitude of external exposure could not be reached in our study and also in Altindag et al. study, which, could be due to limited number of workers studied.

This study had distinctive advantages over similar recent studies, in form of monitoring workers for their external exposure and simultaneous biomonitoring of a group of glass sandblasters with exclusive exposure to crystalline silica respiratory aerosols. Despite of establishing two biomarkers in form of serum malondialdehyde and urinary neopterin simultaneously, further studies on a larger population of glass sandblasters for establishing quantitative rather than qualitative biomarkers with the capability of a better risk assessment for crystalline silica exposure is recommended.
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