Original Article

Evaluation of Serum and Urinary Neopterin Levels as a Biomarker for Occupational Exposure to Crystalline Silica

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Abstract

Background: Crystalline silica is a commonly used mineral in various industries and construction activities, and it is so important introducing potential biomarkers to identify early indicators of biological effects in its high-risk occupational exposures. **Aim:** The present study was aimed to assess the blood and urinary neopterin as an early biomarker of exposure in the workers of an insulator manufacturing plant who are exposed to crystalline silica. **Subjects and Methods:** This analytical descriptive study was done among two groups of exposed workers ($n = 55$) and unexposed office workers ($n = 38$) of an insulator manufacturing plant. Statistical software R was used to determine sample size and select the participants by random sampling among nonsmoker workers. Sampling of airborne silica in breathing zone of participants was done based on the National Institute for Occupational Safety and Health method 7601. The urinary and blood samples were collected and prepared for analysis by high-performance liquid chromatography to determine the level of urinary and serum neopterin. All of the statistical analyses were carried out using SPSS 22. **Results:** The airborne silica concentration was significantly different between two exposed and unexposed groups ($P < 0.001$, 0.27 [0.11] vs. 0.0028 [0.0006] mg/m^3, respectively). The urinary neopterin in exposed group is significantly higher than the unexposed one ($P < 0.001$, 97.67 [30.24] vs. 55.52 [2.18] µmol/mol creatinine, respectively). Neopterin level of serum in exposed group is higher than the unexposed group, and there is a significant difference between them ($P < 0.001$, 6.90 [2.70] vs. 2.20 [1.20] nmol/l, respectively). The positive significant correlations were found between silica exposure concentration with urinary and serum neopterin ($P < 0.001$, $r = 0.36$ and 0.59, respectively). **Conclusions:** Considering the sensitively and easily measurement of neopterin in biological fluid and also the statistically significant positive relationships which were found between the airborne silica concentration and neopterin levels in the present study, the serum and urinary neopterin levels can be considered the potential biomarkers of silica exposure for doing further comprehensive studies in this area.

Keywords: Airborne, biomarker, crystalline silica, neopterin, occupational exposure

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Introduction

Airborne hazards such as dust, vapors, and fumes are present in many workplaces.[1] The risk of exposure to crystalline silica is a great concern worldwide. The term "silica" refers to the silicon dioxide (SiO_2) found in amorphous (noncrystalline) or crystalline form. As one of the most abundant minerals, silica exists in different materials and in the crust of the earth. Free crystalline silica can be found in three polymorphs including alpha-quartz, cristobalite, and tridymite.[1] While silica is a commonly used mineral in various industries and construction activities, exposure to silica dust can result in respiratory disorders.[2] The health hazards associated with occupational exposure to crystalline silica include silicosis, lung cancer, tuberculosis, chronic bronchitis, kidney disease, tooth abrasion, and autoimmune diseases such as rheumatoid arthritis.[3] Silicosis is classified into three clinical and pathologic varieties including acute, accelerated, and chronic silicosis. The chronic type occurs after 10–20 years, the accelerated one occurs after 5–10 years, and the acute one develops within several weeks to 5 years. As the disease progresses, some symptoms including shortness of breath, severe coughing, fatigue, loss of appetite, chest pain, and fever may happen. The concentration of exposure to silica is a determinative factor for developing silicosis.[4] Workers of various workshops and industries depending on their jobs are at risk to diseases associated with silica dust exposure. Workers engaged in insulator manufacturing are one of them who at risk of silica exposure. An electrical silicon-based insulator is applied as a high electrical resistance structure in power transmission towers, at the junction of cables and tower. During most of its manufacturing processes such as milling, mixing, pressing, assembling, cutting, grinding, and extruding equipment, there is the possibility of emitting silica particles.[5]

The diagnosis of silicosis is usually possible when someone has been involved, and the disease has progressed. Therefore, it is important introducing potential biomarkers to identify early indicators of biological effects before other clinical manifestation in the high-risk occupational exposures. It should be noted that the International Agency for Research on Cancer has classified the silica as a carcinogen agent, causing the lung cancer. Given the fact that the silica is extremely hazardous substances, assessing the silica particles in the workplaces under risk is so critical.[6]

Many workers are exposed to silica particles in the wide variety of occupations so that they are at risk of damage to DNA and lipids peroxidation through oxidative stress.[7] Silicosis can be considered as a chronic inflammation in which activated immune cells secrete toxins, causing damage to the lung tissue and development of the lung cancer.[8,9] Therefore, continuous monitoring of the early manifestations of exposure to silica appeared in workers may provide valuable information about worker's health status, resulting in the prevention of disease progression.[10] Neopterin, a pteridine derivative and a by-product of the guanosine triphosphate-biopterin pathway,[11] is known as a biomarker of oxidative stress resulted in the response of immune system cells to inhaled silica particles.[12,13] Neopterin, as a marker of immune activation, is produced by activated macrophages and is released during or after sepsis, elective surgery, and severe trauma.[14] Studies show that high neopterin levels may be a marker of viral, bacterial, and parasitic infections,[15] a prognostic biomarker in intensive care,[14] and also as a marker in coronary disease activity.[11]

According to literatures, cytokines produced by exposure to crystalline silica lead to activating the immune system and producing the neopterin.[13] Exposure of lung cells to silica particles causes the activation of immune system mechanism and release the neopterin produced by stimulated macrophages and monocytes.[16,17] A study by Altindag *et al*. indicated that the concentration of urinary neopterin in exposed participants was significantly higher than that of the unexposed group, demonstrating the importance of neopterin as a marker of early effects associated with silica exposure.[13] Since the neopterin can be considered a biomarker of exposure to silica and due to the severe health hazards of crystalline silica, the present study was conducted to assess the serum and urinary neopterin in the workers of an insulator manufacturing plant who are exposed to crystalline silica. So by this way, it is possible to add this biological monitoring in periodic medical examinations to prevent the adverse effects resulting from exposure to silica. The purpose of the study was to assess the serum and urinary concentrations of neopterin between exposed and unexposed workers and then to investigate the correlation between workers' exposure to silica and the concentration of neopterin, as a potential biomarker for silica.

Subjects and Methods

Sample size determination

This analytical descriptive study was done among two groups of exposed and unexposed workers of an insulator manufacturing plant from June to August 2015. Statistical software R 3.2.2 (2015; R Development Core Team, Auckland University, New Zealand) was used to determine sample size and select the participants by random sampling among nonsmoker workers. A list of all workers ($n = 181$) involving their history exposure level to silica was provided. To have 90% power to detect 0.70 μg/ml difference between the each exposed group with the unexposed group, when the type I error assumed to be 0.05 and the standard deviation assumed to be 0.65 μg/ml, sample size was calculated to be 18 in each exposed and unexposed groups. As there were five exposed groups which compared with one unexposed group, it was decided to change the ratio to 4-1 in unexposed group compared with each exposed group. In this way, the final sample size has been determined 11 in each exposed group (total = 55) and 44 in unexposed group. Forty-four male healthy and nonexposed office employees to crystalline

silica, who their age and work experience were in accordance with exposed participants, were selected through simple random sampling. There were some data missing including six participants from unexposed group.

A signed informed consent had been obtained from all participants. Workers who have suffered from any infectious disease, autoimmune diseases and other inflammatory diseases, and malignant diseases and were under any other special medical treatment and also have work experience <2-year-old were excluded from the study. Ethical approval was granted by the Research Ethics Committee of Tehran University of Medical Sciences.

Measurement of the concentration of airborne silica

National Institute of Occupational Safety and Health Method 7601[18] was used for sampling of airborne silica in breathing zone of all exposed and unexposed participants to evaluate the status of their respiratory exposure (sampling time: 6–8 h). The collected samples were carried to the laboratory for quantitative analysis. The stock and working solutions prepared according to method were used to plot the calibration curve. To prepare the samples for spectrophotometric analysis, silica standard solutions were filtered through the mixed cellulose ester filters (MCE-37 mm, 0.8 μm; SKC, Pennsylvania, USA). Finally, samples were analyzed by spectrophotometer (Unico SpectroQuest Model SQ2800 Single Beam UV/Visible Scanning Spectrophotometer, Ottawa, Canada).

Measurement of the blood and urinary neopterin levels

Blood and urine samples were taken from all participants in the early morning before work, and then they were transferred into 15 and 50 ml polyethylene tubes, respectively. Samples were stored immediately in an ice box and carried to the laboratory. All blood and urine samples were stored at −20°C until analysis. These biological samples were prepared for analysis by high-performance liquid chromatography (Merck Hitachi model L-7420 HPLC-UV, Midland, ON, Canada, equipped with RP18 column, absorbance wavelength of 353 nm). To analyze the biomarkers in serum and urine samples, calibration standard solutions were prepared, and the calibration curve was plotted based on the obtained data (R^2 = 0.9992, linear dynamic range: 1–2000 ng/ml). Using the obtained model, limit of detection and limit of quantification were computed, 0.0089 and 0.029 ng/ml, respectively. Since the urinary neopterin is reported in terms of urinary creatinine, the creatinine level was also determined by Kinetic Jaffe methods.

Statistical analysis

Microsoft Excel version 2010 (Microsoft Inc., USA) was used to plot the calibration curves. All statistical analyses were carried out by SPSS version 22.00 (Chicago, IL, USA). Descriptive statistics including mean, standard deviation, median, range, frequency, and percentage were used to describe data, and analytical statistics including *t*-test, Mann–Whitney, and analysis of variance (ANOVA) were used to compare the results between the groups. Furthermore, Pearson correlation coefficient is used to measure the strength of a linear association between two variables. Normality of the study data was tested with a one-sample Kolmogorov–Smirnov test. The significance level was set to 0.05.

Results

Table 1 shows some demographic characteristics of study participants. There is no significant difference between the exposed and unexposed groups regarding the age and work experience (*t*-test, $P = 0.89$ and 0.14, respectively).

The time-weighted average concentration of silica for exposed and unexposed participants can be seen in Table 2. The significant difference was found between two groups regarding the silica level exposure ($P < 0.001$). The highest silica concentration (0.36 [0.13] mg/m^3) was related to glazing workers, and the lowest concentration (0.11 [0.04] mg/m^3) was obtained for the workers of subordinate and small parts of the factory [Table 2]. According to results, the exposure concentration of silica was significant difference between workers of all parts of factory and unexposed ones ($P < 0.001$). The concentration of urinary neopterin (μmol/mol creatinine) in both groups is presented in Table 3. The urinary neopterin in exposed group is significantly higher than the unexposed one (Mann–Whitney, $P < 0.001$). According to findings, the highest urinary concentration of neopterin (139.77 [14.95] μmol/mol creatinine) was related to glazing workers, and the lowest one (55.52 [2.18] μmol/mol creatinine) was obtained for unexposed group. The urinary concentration of neopterin was significantly different between exposed and unexposed groups in all parts of factory ($P < 0.001$).

Table 4 indicates the serum level of neopterin (nmol/l) in both groups. Based on results, neopterin level of serum in exposed group is higher than the unexposed group, and there is a significant difference between them (ANOVA, $P < 0.001$). As it can be seen in Table 4, the highest and lowest serum concentrations of neopterin (7.80 [0.20] and 2.20 [1.20] nmol/l) were obtained for glazing workers and unexposed group,

Table 1: Some demographic data of study participants

Demographic variables	Exposed group (*n*=55)			Unexposed group (*n*=38)			*P*
	Minimum	Maximum	Mean (SD)	Minimum	Maximum	Mean (SD)	
Age (year)	23	45	37.29 (3.79)	23	46	37.52 (5.56)	0.89
Work experience (year)	2	18	12.91 (3.74)	4	20	11.47 (4.15)	0.14

respectively. The concentration of neopterin in serum was significantly different between exposed and unexposed groups in all parts of factory ($P < 0.001$).

Table 5 illustrates the relationship of silica concentration, age, and work experience with serum and urinary neopterin. Findings demonstrate that there was no significant correlation between these two these demographic variables and urinary and serum neopterin (Pearson correlation test, $P > 0.06$). The positive significant correlations were found between silica exposure concentration and urinary and serum neopterin ($P < 0.001$, $r = 0.36$ and 0.59, respectively).

Table 2: The time-weighted average concentration of airborne silica in different sections of factory

Section (*n*)	Mean (SD) (mg/m³)	*P**
Glazing (11)	0.36 (0.13)	<0.001
Forming (11)	0.29 (0.11)	<0.001
Furnace (11)	0.35 (0.17)	<0.001
Qualitative control (11)	0.25 (0.10)	<0.001
Others (11)	0.11 (0.04)	<0.001
Total (exposed group) (55)	0.27 (0.11)	<0.001
Total (unexposed group) (38)	0.0028 (0.006)	-

*Mean difference of silica concentration between exposed and unexposed groups. SD: Standard deviation

Table 3: The urinary concentration of neopterin in exposed and unexposed groups

Section	Mean (SD) (μmol/mol creatinine)	*P**
Glazing	139.77 (14.95)	<0.001
Forming	84.79 (5.48)	<0.001
Furnace	102.45 (7.59)	<0.001
Qualitative control	104.34 (4.57)	<0.001
Others	57.01 (2.96)	<0.001
Total (exposed group)	97.67 (30.24)	<0.001
Total (unexposed group)	55.52 (2.18)	-

*Mean difference of urinary concentration of neopterin between exposed and unexposed groups. SD: Standard deviation

Table 4: The serum concentration of neopterin in exposed and unexposed groups

Working parts	Mean (SD) (nmol/L)	*P**
Glazing	7.80 (0.20)	<0.001
Forming	6.80 (0.22)	<0.001
Furnace	7.20 (0.25)	<0.001
Qualitative control	7.30 (0.30)	<0.001
Others	5.30 (0.32)	<0.001
Total (exposed group)	6.90 (2.70)	<0.001
Total (unexposed group)	2.20 (1.20)	-

*Mean difference of serum concentration of neopterin between exposed and unexposed groups. SD: Standard deviation

Table 5: The relationship of age, years of work, and silica level with urinary and serum neopterin

Variables	Urinary neopterin (μmol/mol creatinine)		Serum neopterin (nmol/L)	
	r	*P*	*r*	*P*
Age (year)	0.05	0.62	0.06	0.59
Work experience (year)	0.19	0.06	0.17	0.09
Silica concentration (mg/m³)	0.36	<0.001	0.59	<0.001

Discussion

Crystalline silica is one of the most important minerals in various industrial activities worldwide. The concentration exposure of silica is a determinative factor in the development of silicosis.[5] The diagnosis of silicosis is possible when it has progressed and person has been involved. Therefore, it is important to propose appropriate biomarkers to identify the high-risk exposures.[6] Wachter *et al*. first introduced the neopterin as a marker of immune system activation.[19,20] The present study was conducted to evaluate the blood and urinary level of neopterin as a potential biomarker of silica exposure.

The blood and urinary neopterin levels in workers exposed to silica were compared to those of unexposed participants from an insulator manufacturing plant. According to demographic data, there was no significant difference between the exposed and unexposed groups regarding the variables of age and work experience ($P > 0.14$). The concentrations of airborne silica for exposed and unexposed participants were 0.27 (0.11) mg/m³ and 0.0028 (0.006) mg/m³, respectively ($P < 0.001$). American Conference of Governmental Industrial Hygienists has recommended the threshold limit value of 0.025 mg/m³ for respirable silica crystalline.[21] Yassin *et al*. (2005) conducted a study to determine the occupational exposure to airborne silica for 7206 American workers between 1988 and 2003. The mean of silica concentration was reported 0.77 mg/m³ that it is higher than the mean concentration obtained in the present study.[22]

The highest and lowest value of urinary neopterin was obtained 139.77 (14.95) and 55.52 (2.18) μmol/mol creatinine, respectively, for glazing workers and unexposed group. The measurements of neopterin in serum showed that the maximum level of this variable was 7.80 (0.20) nmol/l for glazing workers and minimum level was 2.20 (1.20) nmol/l for unexposed one. Normal value of neopterin in urine for 26–35-year-old men is 101 (33) μmol/mol creatinine and for 36–45-year-old men is 109 (28) μmol/mol creatinine. Normal value of neopterin in serum for 19–75-year-old people is 5.3 (2.7) nmol/l.[23,24] Increased concentrations of neopterin have been shown that can induce the formation of reactive oxygen and nitrogen species. High level of neopterin can contribute to endothelial injury and risk of infection.[14]

Based on results, a significant increase in the serum and urinary neopterin levels of exposed groups was found. According to the literature, on the one hand, silica has proven to change immunological functions, T-lymphocytes, neutrophils, and immunoglobulin;[13,25,26] and on the other hand, several studies have introduced neopterin as a sensitive marker of cellular

immune activation in humans.[13,27] Therefor, it can be stated that the observed differences in neopterin levels between two exposed and unexposed groups may be related to the effect of crystalline silica on pulmonary cells which is followed by the activation of immune system cells and stimulation of macrophages and monocytes, and subsequently increased secretion of neopterin.

The release of neopterin by macrophages is related to the ability of the cells to produce toxic metabolites, especially reactive oxygen species (ROS). Thus, neopterin is not only a marker for activated cell-mediated immunity but also it is a monitor of oxidative stress as a result of immune activation.[13] Silica exposure can lead to the lung inflammation in which activated immune cells secrete toxins, causing damage to lung tissue.[8,9]

The present finding is in line with Wachter *et al*.[20] A study by Prakova *et al*. demonstrated that the level of serum neopterin in workers exposed to silica was significantly higher than that of the unexposed participants; thus, they introduced the neopterin as a potential marker of effect for crystalline silica exposure.[12]

Results of the present study also indicated that there was no significant relationship for the age and years of work with serum and urinary levels of neopterin. The study of Altindag *et al*. in a foundry industry showed that the age of participants had no significant relationship with serum and urinary levels of neopterin.[13] However, normal values of urinary neopterin in healthy people somewhat rely on age and sex of participants.[23,24]

Determination of neopterin levels which can be of importance in the progression of various diseases such as inflammatory diseases,[24] for example, due to silica exposure, but for making a definitive conclusion about the findings of the present study, the further comprehensive studies are certainly necessary to consider more confounding factors such as existence of any form of cardiovascular disease, renal impairment, sepsis, surgery, and severe trauma. Moreover, to confirm the results, it also needs to monitor the other biomarkers closely associated with neopterin levels such as oxidative stress-related parameters.[13]

The health hazards associated with silica exposure can highly dependent on particle size. Particles in the size range 0.5–5 μm cause a significant and fibrogenic effect of oxidative stress, since they are too small and can reach the alveoli and they are digested by the alveolar macrophages and it resulted in the cellular immune responses.[12] However, silica particle size distribution is not considered in the present study.

High reactivity of crystalline silica is due to surface – SiOH groups which are formed when SiO_2 becomes hydrated. Silica dust trigger producing ROS from alveolar macrophages, which overwhelms antioxidant defenses of the lung and contribute to lipid peroxidation and an increased likelihood of lung injury and DNA damage.[28-30] Therefore, in some studies, plasma/serum malondialdehyde levels as an index of lipid peroxidation, 8-hydroxyldeoxyguanosine as an oxidative stress marker, and erythrocyte reduced glutathione levels as an index of antioxidant status were investigated in silica-exposed participants.[28-30] Moreover, more attention has been given to assay of *in-vitro* DNA strand breakages resulting from the biologic interactions of oxygen radicals generated by silica particles.[30,31] Gulumian *et al*. (2006) performed a comprehensive review on suitable biomarkers of silicosis and coal worker's pneumoconiosis and introduced the number of ideal biological markers of them.[32] They concluded that "the determination of serum neopterin levels may be a useful early biomarker following exposure to crystalline silica if combined with other biomarkers."[32]

Conclusion

According to literatures, increased concentration of neopterin in body fluids such as serum and urine can provide useful information about the activation of immune system. High production of neopterin can help identifying and predicting the immunologic changes in some diseases. Findings of this study indicated that there are the statistically significant positive relationships between the silica concentration and serum and urinary neopterin among participants, so higher exposure level to silica displayed higher this biomarker values.

Neopterin is a biologically stable biomarker, and the measurement of neopterin in human biological fluids can be sensitively and easily performed. Furthermore, it has been also found to be a strong predictor of disease progression,[14] so it can be suggested as a potential indicator for determining the early health effects resulted from silica exposure. However, further studies in larger populations are recommended for more accurate assessment of the risk threatening workers exposed to crystalline silica.

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Conflicts of interest

There are no conflicts of interest.

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