Original Article

Potential in the Diagnosis of Oxidative Stress Biomarkers in Noninvasive Samples of Urine and Saliva and Comparison with Serum of Persons Exposed to Crystalline Silica

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Abstract

Background: Prolonged exposure to crystalline silica (CS) (SiO_2) dust enhances the production of reactive oxygen species. In many studies, oxidative stress has been measured in the serum of workers exposed to SiO_2 dust. **Aims:** We investigated the body fluids such as urine, saliva, and serum, which can provide very good results for assessing the health status of workers' exposures to SiO_2 dust. **Materials and Methods:** The oxidative stress biomarkers were evaluated in serum, urine, and saliva of 21 workers who were exposed to SiO_2 silica crushing factories in the Hamadan city at the west of Iran as a case group and 28 controls. **Results:** The level of malondialdehyde in serum, urine, and saliva was significantly higher than that in case group compared to controls (22.19 ± 8.70, 9.86 ± 5.43, and 9.41 ± 7.31 nmol/L vs. 7.30 ± 2.22, 6.79 ± 3.21, and 3.93 ± 3.73 nmol/L, respectively). In addition, the total antioxidant capacity in urine (0.23 ± 0.06 vs. 0.29 ± 0.08 mmol/L), as well as catalase in the serum and saliva of case group was lower than that compared to control group (5.46 ± 1.56 and 1.32 ± 0.55 IU/L vs. 12.55 ± 5.72 and 2.32 ± 1.53 IU/L, respectively). **Conclusions:** The current study indicated that chronic exposure to SiO_2 affects significantly on the oxidative stress biomarker levels in serum, urine, and saliva in persons exposed. Furthermore, SiO_2 leads to the induction of oxidative stress and decreases the activity of the antioxidant enzyme.

Keywords: Crystalline silica, oxidative stress, saliva, serum, urine

Introduction

Silica is the most abundant mineral in the earth and a basic component of soil, sand, and rocks, including granite and quartzite. Crystalline (free silica) and amorphous silica are two types of inorganic form of silica.[1] It occurs most commonly in a crystalline form.[2] CS (SiO_2) crust has various industrial applications. Respirable CS (RCS) has been classified as a carcinogen in its quartz or cristobalite forms by the National Institute for Occupational Safety and Health (NIOSH) and International Agency for Research on Cancer.[1] The health effects of exposure to silica have drawn public health concern globally.[3] Occupational exposure to RCS is a serious but preventable health hazard. Long-time exposure to RCS has long been known to cause one of the oldest known industrial diseases, silicosis, and it has been observed that there is a greater risk in workers exposed to very fine particles of CS, as found in quartz and cristobalite flours.[4] More recent studies of occupational exposure to RCS have reported some

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disease including chronic obstructive pulmonary disease, lung cancer,[5,6] some autoimmune diseases,[7,8] and nonmalignant renal disease[9,10] in workers.

Despite the many efforts of the International Labor Organization to control the exposure of workers to SiO_2, millions of workers in industries in various countries are still suffering from this issue, and silicosis, as one of the important effects of exposure to silica, is still one of the health threat in the world.[11] In some studies, the geometric mean of workers' exposure to SiO_2 in stone milling and cutting, foundry, glass manufacturing, sand and gravel mining, sand blast, ceramics, bricks, cement manufacturing, and other similar factories was higher than the threshold limit value recommended by some organizations such as the American Conference of Governmental Industrial Hygienists and NIOSH.[12,13]

Evaluating the individual exposure of workers to harmful agents such as silica is one way to proceed to control occupational diseases. When workers have an unacceptable exposure to harmful agents such as silica, their risk of disease increases. Biological monitoring (BM) is a useful method to assess the exposure of workers to chemicals in the workplace by measuring appropriate biomarkers in biological samples (urine, blood, saliva, etc.). BM can help control and reduce the exposure to chemicals and thus reduce the toxicity caused by exposure. However, until now, no valid and known biomarker has been introduced for the early diagnosis and measurement of silicosis' progress.[14,15]

SiO_2 are important occupational hazards in a wide variety of occupations, and respirable exposure to SiO_2 may result in the production of reactive oxygen species (ROS).[11] A free radical is a group of atoms containing at least one unpaired electron. Unpaired electrons are highly reactive and capable of interacting with other molecules.[16] Oxidative stress due to the increased generation of ROS plays a key role in the pathogenesis and complications of many diseases.[17] Recently, oxidative stress biomarkers have been considered by the researchers in the mechanism of silicosis to find a biochemical marker. Findings suggest that SiO_2 particles can be phagocytized by lung macrophages, which activate the generation of ROS.[18] Because of the key role of SiO_2 in the generation of ROS, this article investigated "the body fluids such as urine, saliva, and serum in workers exposed to SiO_2 dust," while in many of the studies, oxidative stress has only been measured in the serum sample. Thus, the study can provide excellent results for assessing the health status of workers' exposures to SiO_2 dust.

Materials and Methods

Subject selection

In this case–control study, 21 male workers in the Hamadan city who were exposed to SiO_2 in crushing factories participated as a case group. In crushing factories, silica stones are milled, powdered, and granulated, and then are supplied to the industry as Grade 1 and Grade 2 silica. The control group comprised 28 male workers who worked in the food distribution industry without exposure to CS, who were matched for age, duration of exposure, and smoking habit. We explained the purpose of the study to the workers and those unwilling to continue the study were excluded; those who were willing participated in the study with informed consent. The controls were healthy and did not consume drugs. The lifestyle and diet of the included participants were similar. The principles of the Research Ethics Committee of Hamadan University of Medical Sciences (IR.UMSHA.REC.1396.787) were followed during the entire study.

Sample collection

After assuring the satisfaction of the workers, 5 ml of fasting blood samples was taken from the participants in the study by the laboratory staff at the beginning of the shift work. Then, their serums were separated and coded.In addition, urine and salivary samples were collected from all participants and coded. The samples were kept away from direct light and stored at −20°C until analysis.[19]

Biochemical assays

Measurement of malondialdehyde level

Contents of malondialdehyde (MDA) in serum, urine, and saliva were measured spectrofluorometrically as thiobarbituric acid (TBA) reactive substances. TBA reacted with MDA and formed TBA reactant substances, as biomarkers of oxidative damage to polyunsaturated fatty acids and measured at 532 nm by a spectrophotometer.[20]

Measurement of total antioxidant capacity level

The total antioxidant capacity (TAC) was measured using the ferric-reducing ability of serum (FRAP) method. The complex among Fe_2+ and tripyridyltriazine was composed, and the maximum absorption of the produced bluish complex at a wavelength of 593 nm was measured.[21]

Measurement of catalase activity

Catalase (CAT) activity in serum, urine, and saliva was determined using an assay kit according to the instructions of the company (ZellBio GmbH, Veltlinerweg 29, 89075 Ulm, Germany).

Statistical analysis

All values were expressed as means ± standard deviation (SD), and $P < 0.05$ was considered a statistically significant difference. Statistical analysis was done using *t*-test (SPSS version 16, Chicago, Ill, USA) and Graph Pad Prism version 6 (Graph Pad Software, San Diego, CA, USA).

Results

Demographic parameters of the participants are presented in Table 1. The variables such as age (mean ± SD), smoking habit, and duration of work had no statistically significant difference between the case and control groups.

Concentration of malondialdehyde in the study groups

The level of MDA (nmol/L) as an oxidative stress biomarker was evaluated in serum, urine, and saliva in the case group compared with that of the control group.

Table 1: Comparison of demographical data between case and control groups

Demographical data	Subject group		*P**
	Case group (*n*=21)	Control group (*n*=28)	
Age (years), mean±SD	37.14±7.00	33.50±5.31	NS
Duration of work (years), mean±SD	9.28±3.14	7.42±5.42	NS
Smoking habit, *n* (%)			
Yes	17 (80)	24 (85)	NS
No	4 (19)	4 (14)	NS

Data were not significant (NS). *(*P*>0.05), SD: Standard deviation

As is depicted in Figure 1, significant increases were observed in the MDA levels in the case group versus the control group in serum and saliva in the three studied matrixes ($P < 0.05$).

Concentration of total antioxidant capacity in the study groups

As shown in Figure 2, concentrations of TAC (mmol/L) in urine were statistically significantly lower in the case group when compared to that of the control group ($P < 0.05$). However, no statistical difference was found in the TAC level in serum and salivary samples ($P > 0.05$).

Activity of catalase in the study groups

In serum and salivary samples, significant differences were observed in CAT (IU/L) activity regarding antioxidant biomarkers. CAT activity in urine was statistically significantly lower in case group compared to that of the control group ($P < 0.05$) [Figure 3]. However, no statistical difference was found in the CAT activity in the urine samples ($P > 0.05$).

Relationship between ages, duration of exposure, and smoking habit with oxidative stress

The relationship between ages, duration of work, and smoking habit with oxidative stress factors was studied. The results showed a statistically significant negative correlation between age and plasma TAC level ($r = -0.295$, $P = 0.04$) and an inverse correlation between smoking and CAT in saliva ($P = 0.007$). In other cases, there was no significant relationship in the case and control groups.

Discussion

SiO_2 is an important material for industries, but inhaled crystalline SiO_2 particles increase oxidative stress and contribute to serious diseases. In this study, we focused on the lipid peroxidation, TAC, and CAT activities in serum, urine, and saliva of the case (crushing factory workers) and control groups. Interestingly, the results of this study showed increases in oxidative biomarkers such as MDA in serum, urine, and saliva in workers exposed to SiO_2 dust compared to the control group. On the other hand, exposure to SiO_2 dust also reduces antioxidant biomarkers such as CAT activity and TAC levels. Concordant with these findings, previous research shown that SiO_2 dust-induced oxidative toxic stress in persons exposed to

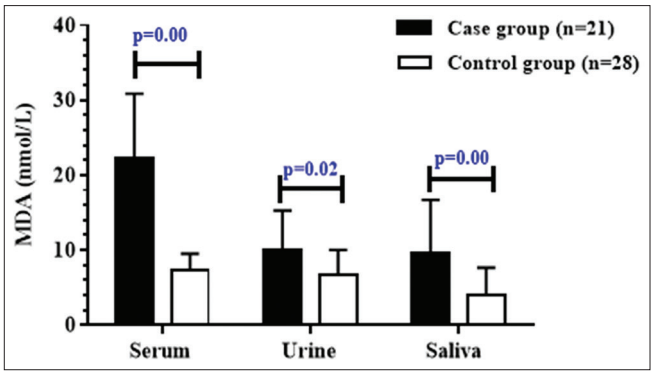

Figure 1: Malondialdehyde concentrations in serum, urine, and saliva of case and control groups. Results are represented as mean ± standard deviation

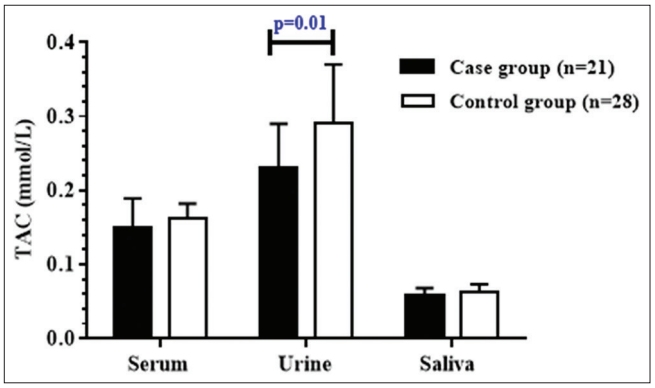

Figure 2: Total antioxidant capacity in serum, urine, and saliva of case and control groups. Results are represented as mean ± standard deviation

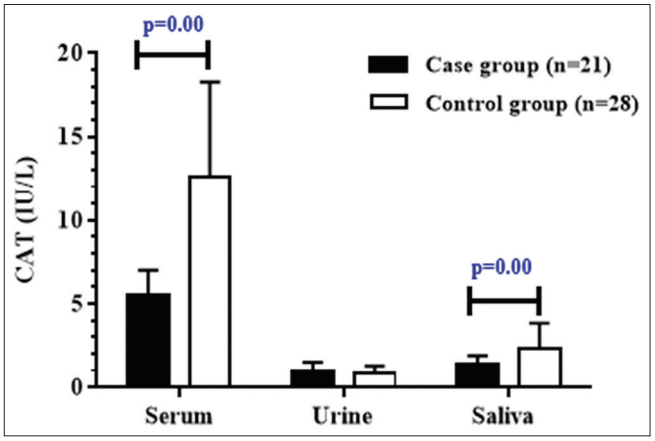

Figure 3: Catalase activity in serum, urine, and saliva of case and control groups. Results are represented as mean ± standard deviation

crystalline silica. The study of Vallyathan *et al*. showed that exposure to a SiO_2 particle resulted in an increased generation of ROS with enhanced levels of oxidative biomarkers such as MDA in rat lung in *in vivo* experiments.[22] Silica particles react with the cell membrane, leading to peroxidation of membrane lipids and damage to cell membranes.[23] In workers with cement dust-exposed silicosis, increased plasma MDA levels were reported by Orman *et al*.[24] Azari *et al*. investigated lipid

peroxidation in glass sandblaster workers exposed to SiO_2 aerosols through measuring serum MDA levels and indicated that serum MDA could be accepted as an indicator of oxidative toxic stress in workers exposed to SiO_2 aerosols.[11] It has been shown that in the presence of water, SiO_2 becomes hydrated to form silanol groups (–SiOH) that react with scavenger receptors on macrophages and activate the production of oxidative toxic stress and inflammatory cytokines.[25]

It was recently shown that inhalation of SiO_2 reduced superoxide dismutase (SOD) activity as an antioxidant enzyme that these changes are consistent with the results of this research.[26] In addition, Fubini and Hubbard have shown that SiO_2 can produce oxidative toxic stress by the activation of cell signaling pathways including MAPK/extracellular signal-regulated kinase (ERK) kinase, upregulation of inflammatory cytokines (e.g., tumor necrosis factor alpha and interleukin-1), ERK phosphorylation, and activation of specific transcription factors (e.g., NF-κB and AP-1).[27] Pournourmohammadi *et al*. demonstrated that serum levels of total thiol molecules (TTM) and TAC were significantly lower among cement plant workers in comparison to healthy controls.[18]

The present study showed a significantly inverse correlation between age and plasma TAC concentration and smoking and CAT in saliva, whereas in other cases, we did not find a correlation between case and control groups. He *et al*. showed that levels of serum glutathione (GSH), MDA, and SOD activity were not associated with age among both the controls and silicosis patients.[28] In addition, Kamal *et al*. demonstrated a nonsignificance between ages and duration of silica exposure with plasma MDA levels.[29] On investigating the effects of occupational silica exposure on oxidative stress and immune system parameters, Anlar *et al*. showed that there was no significant correlation between GSH levels and activities of GR, CAT, and SOD with age, smoking, and duration of exposure.[30] In the study of oxidative stress biomarkers in ceramic workers compared to a control group, the results showed a nonsignificant oxidative stress index measured with an increase in years of work.[31] Nielsen *et al*. revealed an association between plasma MDA and the number of hours of daily exposure to cigarette smoke, but they found no clear correlation between plasma MDA and the number of cigarettes smoked.[32]

Therefore, SiO_2 can produce ROS, and thus due to the known effects of oxidative injury in the progress of silicosis, administration of antioxidant agents may be a beneficial way to prevent the progression of illness caused by silica. Furthermore, as mentioned, we investigated the body fluids such as urine, saliva, and serum in workers exposed to SiO_2 dust. Although examining oxidant–antioxidant status in serum is a standard practice, but biological specimens such as urine, saliva and other non-invasive samples can be better sample than serum to diagnose and track of clinical disorders. As mentioned, saliva provides noninvasiveness and stress-free sample collection, easy and multiple sampling opportunities, decreased need for sample preprocessing, and low risk of contracting infectious organisms, and it is also an ideal biofluid for collecting samples from patients.[33] In addition, urine specimen is one of the most attractive and useful specimens for routine testing, which provides an excellent resource for the analysis of many biomarkers such as oxidative stress status, with the advantage over tissue biopsy specimens due to the ease and noninvasive nature of the collection. Therefore, saliva and urine specimens could be used in clinical practice for monitoring and diagnosis of many disease markers.

Conclusions

Our results show that exposure to SiO_2 dust related to increased levels of free radicals in serum, urine, and saliva. SiO_2 increases the level of MDA and decreases the levels of TAC and CAT in samples from the case group. This suggests that exposure to SiO_2 dust causes impairment in serum, urine, and saliva oxidant–antioxidant status. Further studies are required to comprehend the health influences and mechanisms due to human exposure to SiO_2 dust.

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Conflicts of interest

There are no conflicts of interest.

References

1. Platias S, Vatalis KI, Charalampides G. Suitability of Quartz Sands for Different Industrial Applications. Procedia Economics and Finance 2014;14:491-8.
2. Brown T. Silica exposure, smoking, silicosis and lung cancer-complex interactions. Occup Med (Lond) 2009;59:89-95.
3. Güngen AC, Aydemir Y, Çoban H, Düzenli H, Tasdemir C. Lung cancer in patients diagnosed with silicosis should be investigated. Respir Med Case Rep 2016;18:93-5.
4. Pilkington A, Maclaren W, Searl A, Davis J, Hurley J, Soutar C. Scientific Opinion on the Health Effects of Airborne Crystalline Silica. Edinburgh: Institute of Occupational Medicine; 1996.
5. Mannetje A, Steenland K, Attfield M, Boffetta P, Checkoway H, DeKlerk N, *et al*. Exposure-response analysis and risk assessment for silica and silicosis mortality in a pooled analysis of six cohorts. Occup Environ Med 2002;59:723-8.
6. Sanjel S, Khanal SN, Thygerson SM, Carter W, Johnston JD, Joshi SK. Exposure to respirable silica among clay brick workers in Kathmandu valley, Nepal. Arch Environ Occup Health 2018;73:347-50.
7. Conrad K, Mehlhorn J, Lüthke K, Dörner T, Frank KH. Systemic lupus erythematosus after heavy exposure to quartz dust in uranium mines: Clinical and serological characteristics. Lupus 1996;5:62-9.
8. Brown LM, Gridley G, Olsen JH, Mellemkjaer L, Linet MS, Fraumeni JF Jr., Cancer risk and mortality patterns among silicotic men in Sweden and Denmark. J Occup Environ Med 1997;39:633-8.

9. Calvert GM, Steenland K, Palu S. End-stage renal disease among silica-exposed gold miners. A new method for assessing incidence among epidemiologic cohorts. JAMA 1997;277:1219-23.
10. Steenland K, Rosenman K, Socie E, Valiante D. Silicosis and end-stage renal disease. Scand J Work Environ Health 2002;28:439-42.
11. Azari MR, Ramazani B, Mosavian MA, Movahadi M, Salehpour S. Serum malondialdehyde and urinary neopterin levels in glass sandblasters exposed to crystalline silica aerosols. Int J Occup Hyg 2011;3:29-32.
12. Normohammadi M, Kakooei H, Omidi L, Yari S, Alimi R. Risk assessment of exposure to silica dust in building demolition sites. Saf Health Work 2016;7:251-5.
13. Pavilonis BT, Mirer FE. Respirable dust and silica exposure among World Trade Center cleanup workers. J Occup Environ Hyg 2017;14:187-94.
14. Leese E, Staff JF, Carolan VA, Morton J. Exhaled breath condensate: A novel matrix for biological monitoring to assess occupational exposure to respirable crystalline silica. Ann Work Expo Health 2017;61:902-6.
15. Sakhvidi MJ, Biabani Ardekani J, Firoozichahak A, Zavarreza J, Hajaghazade M, Mostaghaci M, *et al*. Exhaled breath malondialdehyde, spirometric results and dust exposure assessment in ceramics production workers. Int J Occup Med Environ Health 2015;28:81-9.
16. Kheiripour N, Karimi J, Khodadadi I, Tavilani H, Goodarzi MT, Hashemnia M. Silymarin prevents lipid accumulation in the liver of rats with type 2 diabetes via sirtuin1 and SREBP-1c. J Basic Clin Physiol Pharmacol 2018;29:301-8.
17. Ranjbar A, Firozian F, Soleimani Asl S, Ghasemi H, Taheri Azandariani M, Larki A, *et al*. Nitrosative DNA damage after sub-chronic exposure to silver nanoparticle induces stress nephrotoxicity in rat kidney. Toxin Rev 2018;37:327-33.
18. Pournourmohammadi S, Khazaeli P, Eslamizad S, Tajvar A, Mohammadirad A, Abdollahi M. Study on the oxidative stress status among cement plant workers. Hum Exp Toxicol 2008;27:463-9.
19. Syslová K, Kacer P, Kuzma M, Najmanová V, Fenclová Z, Vlcková S, *et al*. Rapid and easy method for monitoring oxidative stress markers in body fluids of patients with asbestos or silica-induced lung diseases. J Chromatogr B Analyt Technol Biomed Life Sci 2009;877:2477-86.
20. Zanganeh N, Siahpoushi E, Kheiripour N, Kazemi S, Goodarzi MT, Alikhani MY. Brucellosis Causes Alteration in Trace Elements and Oxidative Stress Factors. Biol Trace Elem Res 2018;182:204-8.
21. Ranjbar A, Kheiripour N, Ghasemi H, Seif Rabiei MA, Dadras F, Khoshjou F. Antioxidative Effects of Tempol on Mitochondrial Dysfunction in Diabetic Nephropathy. Iran J Kidney Dis 2018;12:84-90.
22. Vallyathan V, Leonard S, Kuppusamy P, Pack D, Chzhan M, Sanders SP, *et al*. Oxidative stress *in silico*sis: Evidence for the enhanced clearance of free radicals from whole lungs. Mol Cell Biochem 1997;168:125-32.
23. Jalali M, Zare Sakhvidi MJ, Bahrami A, Berijani N, Mahjub H. Oxidative Stress Biomarkers in Exhaled Breath of Workers Exposed to Crystalline Silica Dust by SPME-GC-MS. J Res Health Sci 2016;16:153-61.
24. Orman A, Kahraman A, Cakar H, Ellidokuz H, Serteser M. Plasma malondialdehyde and erythrocyte glutathione levels in workers with cement dust-exposure [corrected]. Toxicology 2005;207:15-20.
25. Aydin S, Aral I, Kilic N, Bakan I, Aydin S, Erman F. The level of antioxidant enzymes, plasma vitamins C and E in cement plant workers. Clin Chim Acta 2004;341:193-8.
26. Miao RM, Zhang XT, Guo P, He EQ, Zhou F, Zhao DK, *et al*. Effect of oxidative stress on development of silicosis. World J Respirol. 2012;2:1-5.
27. Fubini B, Hubbard A. Reactive oxygen species (ROS) and reactive nitrogen species (RNS) generation by silica in inflammation and fibrosis. Free Radic Biol Med 2003;34:1507-16.
28. He JL, Zhang JW, Lv GC, Zhao Y, Hong XP. Study on the serum oxidative stress status *in silico*sis patients. Afr J Biotechnol 2011;10:10504-8.
29. Kamal AA, Gomaa A, el Khafif M, Hammad AS. Plasma lipid peroxides among workers exposed to silica or asbestos dusts. Environ Res 1989;49:173-80.
30. Anlar HG, Bacanli M, İritaş S, Bal C, Kurt T, Tutkun E, *et al*. Effects of Occupational Silica Exposure on OXIDATIVE Stress and Immune System Parameters in Ceramic Workers in TURKEY. J Toxicol Environ Health A 2017;80:688-96.
31. Shad KM, Barkhordari A, Ranjbar A, Mehrparvar A, Dehghani A. The study of oxidative stress biomarkers in ceramic workers compared to control group. Occup Med Q J 2015;7:67-81.
32. Nielsen F, Mikkelsen BB, Nielsen JB, Andersen HR, Grandjean P. Plasma malondialdehyde as biomarker for oxidative stress: Reference interval and effects of life-style factors. Clin Chem 1997;43:1209-14.
33. Rahim MA, Rahim ZH, Ahmad WA, Hashim OH. Can saliva proteins be used to predict the onset of acute myocardial infarction among high-risk patients? Int J Med Sci 2015;12:329-35.