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Evaluation of oxidative stress biomarkers and liver enzyme activity in workers occupationally exposed to respirable free crystalline silica

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ABSTRACT

Introduction: In various mining activities, workers are exposed to free Crystalline Silica (CS), which can cause the constant production of reactive oxygen species and silicosis. This research was conducted to evaluate oxidative stress biomarkers and liver tissue function in workers occupationally exposed to CS during their activities.

Materials and methods: In this study, the biomarkers of oxidative stress were evaluated in 40 workers in silica mines of Azandarian region (Hamadan province, Iran) with occupational exposure to CS, as the case group and 40 workers without any silica-exposure as controls.

Results: A significant higher serum levels of Malondialdehyde (MDA), Reactive Oxygen Species (ROS) and Alanine Transaminase (ALT) were observed in the silica-exposed group compared to the controls. Moreover, in the serum of the silica-exposed cases, the total antioxidant capacity was lower than that of the control group. Based on findings chronic exposure to CS can obviously affect the serum levels of oxidative stress biomarker and liver tissue function in the exposed workers.

Conclusion: Therefore, the use of suitable face mask and different dietary antioxidants are recommended in the silica-exposed workers for the reduction of oxidative stress production and prevention of liver tissue disorders.

Introduction

Repairable Crystalline Silica (RCS) as a common mineral found in the earth's crust can be considered an environmental and occupational pollutant. RCS exposure may occur in industrial and non-industrial sources [1]. From non-industrial sources exposures can point to mining and grinding of sandstone, ceramic, silica grinding, granite and quartz crushing that have many effects the human health [2]. The non-industrial source exposures are naturally occurred in the environment such as sandstorms in deserts, which are harmful to health

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[3]. In developing countries, there are active silica crushing units that cause chronic exposure to RCS in the workplaces [4]. Although many efforts accomplished to control the exposure of workers to RCS, millions of workers in various industries are occupationally exposed to RCS at their workplaces [5].

It has been reported that occupational exposure to RCS is associated with a wide range of disorders such as pulmonary obstructive, silicosis, pulmonary fibrosis, chronic bronchitis, kidney diseases and Systemic Autoimmune Rheumatic Disease (SARD) [6]. The International Agency for Research on Cancer (IARC) and the World Health Organization (WHO) have reported that 3.2 million workers are exposed to RCS in the European Union and have classified this compound as carcinogenic to humans [3, 7] Although the lung is the main target of damage due to chronic exposure to silica dusts (RCS), many studies have shown that prolong exposure to this compound is associated with a wide range of adverse effects on other organs [6]. Although the molecular mechanisms in the pathogenesis of silicosis are still not entirely known, accumulating evidence suggested that the phagocytosis of RCS causes an active inflammation and oxidative stress that plays an important role in the various diseases in the exposed individuals.

Depending on the duration and exposure concentration of silica dusts, silicosis can occur as acute, progressive, chronic, and conglomerate silicosis (advanced progressive silicosis or complex silicosis) [8]. After exposure to RCS, the dusts are able to reach the alveoli, inducing oxidative stress by the formation of Reactive Oxygen (ROS) and Nitrogen Species (RNS) [9]. As mentioned abundantly excess generation of free radicals can eventually lead to the damage of cellular lipids, proteins, and DNA, inhibiting their functions and also decrease of antioxidant enzymes such as Glutathione S-Transferase (GST), Catalase (CAT), Superoxide Dismutase (SOD) as first line of defense against free radical [10].

The many studies have conducted on the assessment of health risk of exposure to RCS in workers of various occupations, including coal miners, pottery, cement, stone crushers and granite foundry [3, 10, 11]. For example, some researchers indicated the workers working in high-risk units such as stamping machine operator and stone separation operator are more likely to suffer from adverse health complications such as silicosis, lung cancer and other respiratory complications [3]. Other researchers concluded exposure of humans to cement dust is capable of inducing free radicals, marked hazardous alterations in some enzymatic activities, liver functions and some biochemical parameters [10]. Therefore, given the actual exposure of workers to RCS in silica mines of Azandarian region (Hamadan province, Iran) and the lack of a determined method for early detection and screening of high risk workers before occurring silicosis, the present study aimed to evaluate the biochemical parameters of liver enzyme activity and oxidative stress as potential early peripheral biomarkers for investigating the health status of the silica-exposed workers in silica mines.

Materials and methods

Human subjects

The current case–control study was performed in different parts of industrial production units of silica in Azandrian region, Hamadan province in West of Iran, during spring, 2021. In this study, in the first step, all workers in various units of silica production agreed to participate in the study and were asked to sign a consent form. Next, all workers were asked to complete a questionnaire containing health status and demographic information including income, education, lifestyle, smoking, alcohol consumption, intake of drug, or/and supplement and diet. Moreover,

to identify any type of chronic diseases include (diabetes, blood pressure, cardiovascular diseases, chronic kidney disease, immune system disorders, cancer, respiratory diseases including bronchitis, epilepsy, hearing loss, digestive diseases, asthma, etc.) all the participants were asked to complete clinical examination. All subject with alcohol, chronic diseases and antioxidants less than six months in silica production units were excluded from the study. Therefore, eighty participants were included in the study and classified into two groups, including 40 workers exposed to RCS working in various units of silica production as the case group (or exposed group) and 40 well-matched workers whiteout any exposer to silica dusts who were working in food distribution industry. Current study was approved by the Research Ethics Committee of Hamadan University of Medical Sciences (IR.UMSHA.REC.1400.384). All volunteers provided informed consent prior to participation in this study.

Sample collection

Blood samples from study subjects were drawn by venipuncture. Samples were centrifuged immediately at 800 g for 15 min to obtain plasma and kept in −80 °C for further analyses. All the participants were sampled on the same day. Complete blood analysis was done to determine Alanine Transaminase (ALT), Aspartate aminotransferase (AST) and Alkaline Phosphatase (ALP).

Biochemical analysis

Analysis of chemical biomarkers of liver enzyme activity such as (ALT), (AST) and (ALP) was done using Pars Azmun commerical kits (Tehran,Iran, Cat. No: KSOD91).

Measurement of oxidative and anti-oxidative stress markers in serum

Measurement of malondialdehyde level (MDA) and ROS in serum

The lipid peroxidation was evaluated using spectrofluorometrically as Thiobarbituric Acid (TBA) reactive substances. The reaction of TBA with MDA led to the formation of TBA reactant substances, which can be considered as biomarkers of oxidative at acidic pH and high temperature. The maximum absorbance measurement at 532 nm indicated the MDA level. MDA content was considered as (nmol/L) [12]. Kiazist kit (KTOS96), which works based on oxidation of ferrous iron to ferric iron in the presence of oxidant factors, was used for detecting ROS in serum as another indicator of oxidative stress by. ROS levels were expressed as (mM/L).

Evaluation of total antioxidant capacity (TAC) and CAT in serum

TAC measurement was done manually using ferric-reducing ability of serum (FRAP) method. The method is based on the capacity of sample to reduce Fe^{3+} (Ferric) ions to Fe^{2+} (Ferrule) in the presence of TPTZ (Tripyridyl-s-triazin). The interaction of Fe^{2+} - TPTZ complex produces a blue color. Maximum absorption of optical density was measured at 593 nm [13]. Catalase (CAT) is important antioxidant enzymes. The activities of this enzyme are measured by kiazist kits of KSOD94.

Statistical analysis

To compare qualitative data from the Kolmogorov–Smirnov test was used. For determining normal distribution from Independent t-test and Mann- Whitney were used to compare the mean difference between the RCS exposed and control groups in quantitative data. All data were analyzed using SPSS software (Version 20). The results were presented as mean ± SD and $p<0.05$ was considered as statistically significant.

Results and discussion

Table 1 presents the demographic data of the subjects. Based on findings, there were no statistically significant difference between the case and control groups in term of age, duration of work, weight, place of residence ($p>0.05$).

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

Demographical data	Case	Control	
	Mean±SD	Mean±SD	p-value
age	30.53 ±7.74	33.32 ±8.64	0.076[a]
weight	78.66 ±8.22	78.53 ±9.06	0.927[b]
	N (%)	N (%)	P-value [c]
Residence			
City	55 (69.6)	54 (68.4)	0.863
Village	24 (30.4)	25 (31.6)	
Work experience			
1-5 years	9 (11.4)	16 (20.3)	0.060
6-10 years	15 (19.0)	20 (25.3)	
11-15 years	27 (34.2)	29 (36.7)	
>15 years	28 (35.4)	14. (17.7)	

a. Mann-Whitney, b. Independent t-test, c. Chi-square

Table 2. Comparison of blood factors analyses between exposed workers and controls

	Number	Mean ±SD of Case	Mean ±SD of Control	P-value[a]
AST[b]	80	19.27 ± 7.85	19.27 ± 9.23	0.798
ALT[c]	80	18.82 ± 7.02	16.71 ± 6.05	0.041
Alp[d]	80	221.77 ± 57.13	237.04 ± 53.37	0.125

a.Mann- Whitney, d. Aspartate aminotransferase, c. Alanine aminotransferase,

d. Alkaline phosphatase

The concentration of ALP, ALT and AST in serum

As shown in Table 2, blood factors analyses showed a significant difference (increasing trend) in the ALT levels between the exposed cases and controls ($p<0.001$), while no significant difference was observed in term of other blood factors (e.g. ALP and AST) ($p>0.05$).

Concentration of MDA and ROS in serum of the exposed worker

In this step, the serum levels of MDA (nmol/L) and ROS (mM/L), as an oxidative stress biomarkers were measured and compared between the exposed and non-exposed workers. As shown in Fig. 1, there were significant increases in the ROS and MDA levels in the exposed workers compared to the control group ($p<0.01$ and $p<0.001$, respectively).

Concentration of TAC and CAT in serum of the study groups

As shown in Fig. 2 concentrations of TAC (mmol/L) in serum 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 CAT level in serum samples of groups ($P>0.05$).

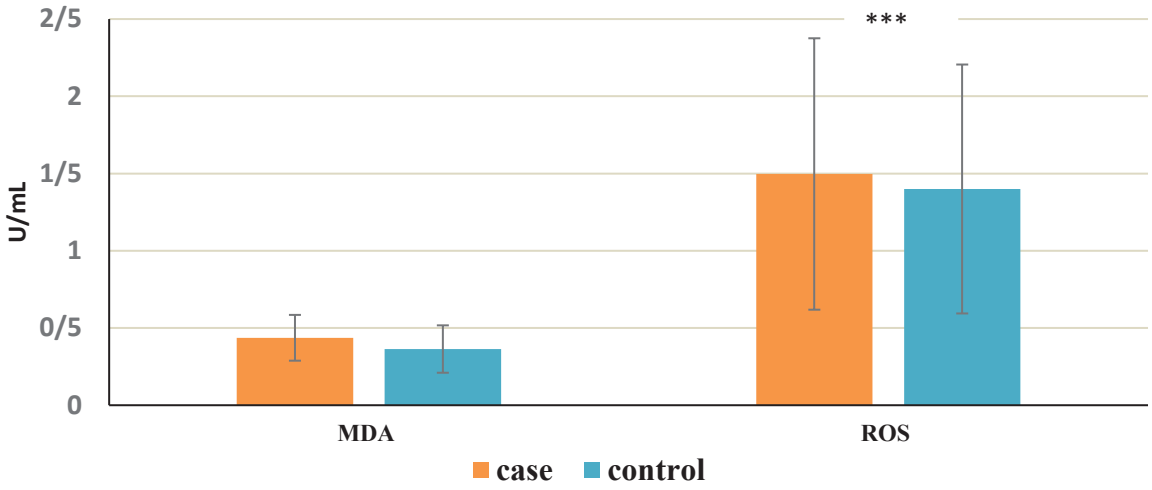

Fig. 1. Serum levels of MDA and ROS in the exposed workers and controls (as mean±standard deviation). Difference between exposed and control groups was considered significant at p-value<0.001 (***), p<0.01(**)

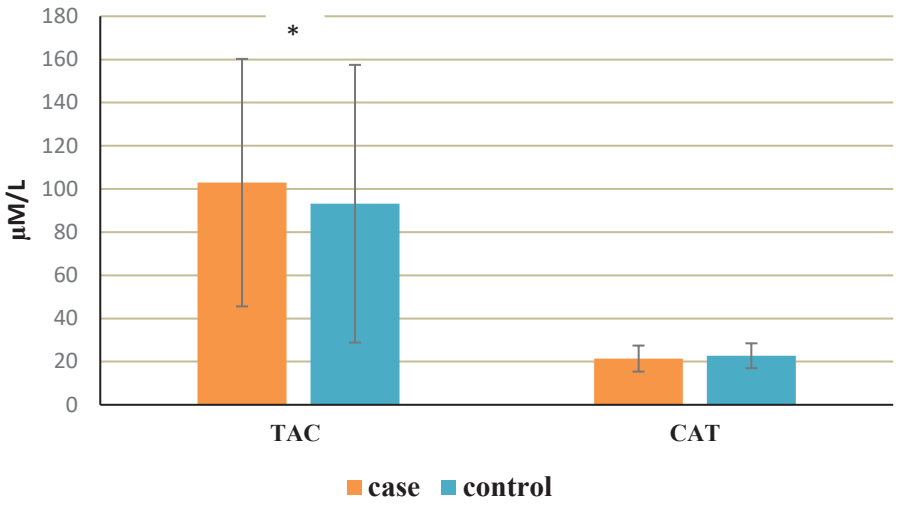

Fig. 2. Concentration of TAC and CAT in serum of case and control groups, Results is represented as mean±standard. Difference between control and other groups is significant at p-value<0.05 (*)

RCS is an important material that is widely used in a wide range of industries and is associated with many health problems in different organs. In this study, we focused on biomarkers of oxidative stress and liver function in serum of the exposed-workers in various units of silica mines of Azandarian region (Hamadan province, Iran) and their results were compared with non-exposed group as controls. Silicosis as an occupational respiratory disorder is generally occurred due to chronic exposure (occupational exposure) RCS.

It is noteworthy that several studies in recent years have reported several silicosis cases with non-occupational exposure. It is described chiefly by huge pulmonary fibrosis due to proliferation and progressive increase in connective tissue, which is substituted for normal functional parenchyma [14]. Although the main target tissue in toxicity with silica crystalline is the lung, but abnormal of blood cells, hematologic injury, breakage of chromosomal, complications including liver dysfunction, cancer and reduced in mitotic index are still the topic of various studies [15, 16]. RCS particles penetrate the worker's organs mainly through the respiratory and skin routes and rarely through ingestion. [17]. Until now, the mechanism underlying the induction of fibrosis and silicosis of lung tissue has been severally studied.

Evidence shows that stress oxidative damage plays a key role in developing fibrotic lung disease, and hence maybe used as an indicator [18]. Our study clearly indicated the function of liver enzyme (ATL level) was significantly decreased in the silica-exposed group compared to the control, while no significant changes were observed in other liver enzymes (ALP and AST) (as can be seen in Table 2). Liver tissue is one of the most important organs that play an important role in the detoxification of endogenous and exogenous compounds in the body. Serum levels of liver enzymes are identified as an estimation of liver function [19]. ALT enzyme is known as a serum dysfunctional enzyme due to the hepatic cell injury [20], while AST enzyme is biomarker of liver tissue damage and is abundantly found in heart, kidney, skeletal muscle and liver [21]. Moreover, ALP is typically concentrated in liver and plays a key role in the detoxification, metabolism and biosynthesis of energetic macromolecules for various essential functions [22]. During cellular degeneration or destruction of liver, these enzymes are released from the cytosol into the bloodstream [10]. Consistent with our results, many researchers, indicated that in silica-exposed workers (in cement factories), there was an association between the level of liver enzymes and the duration of exposure [15]. Other researchers stated that ALT level was higher among cement exposed workers compared with the control group, but no statistically significant [17]. In a study, it was showed that the activities of AST , ALP and ALT in plasma of exposed workers in cement factory had an increase compared to the control group [10]. Moreover, a significant increase can occur in the activity of ALP in plasma due to the change in permeability of plasma membrane or cellular necrosis [23].

According to published studies, oxidative stress occurs due to the imbalance between oxidant and antioxidant status in the body [24]. A large body of experimental work indicated exposure with RCS can induce ROS on epithelial cells and extracellular components (macrophages) and lead to activation of cell signaling pathways, specific transcription factors and increased oxidative stress biomarkers [25]. ROS are active molecular species containing oxygen, such as superoxide anion (O^{2-}), Hydroxyl radical ($HO^{\bullet}$) and Hydrogen peroxide (H_2O_2), which offer a higher chemical activity compared with molecular oxygen [26]. Various antioxidant systems are present in the human body to protect against destructive effects from activated

Reactive Oxygen Species (ROS). As mentioned in previous studies, O^{2-} generation can convert to H_2O_2 by SOD and then by CAT enzymes alter to oxygen and water [27].

Considering the important role of oxidative stress, evaluation of biomarkers of oxidative stress and its relationship with liver function in RCS-exposed workers can help in early diagnosis of silicosis. In our study, an increase was observed in oxidant stress biomarkers (MDA and ROS) and a decrease in the antioxidant system parameters (TAC) in the exposed workers, while there was no significant difference in serum level of CAT in the silica-exposed workers. Prolonged exposure with silica crystals might cause ROS production and membrane lipid peroxidation. Corroborating our results, several studies reported the involvement of lipid peroxidation in plasma, after crystalline silica exposure. In two studies, it was indicated an significant increase in MDA plasma levels of workers in the field of ceramics and glass sandblasters exposed to RCS compared to the non-exposed workers, respectively [28, 29]. Other researchers indicated statistical change in plasma MDA levels cement-exposed workers than control group [30]. In a study, it was found that plasma concentration of MDA were much higher in asbestos-exposed workers compared to non-exposed individuals [31].

CAT enzymes have important role in the detoxification of superoxide anion and hydrogen peroxide (H_2O_2), which protect the cell against ROS-induced injury [30]. This enzyme, along with Total Antioxidant Capacity (TAC) constitutes a major defense against oxidative damage. TAC as valuable antioxidant can be useful as an early marker of oxidative stress to monitor antioxidant status in body. TAC indicate an important index of the total amounts of enzymatic and non-enzymatic antioxidants in body which can the activity of one or two enzymes, and also be various dietary antioxidants such as ascorbic acid and a-tocopherol [32]. Our research indicated a statistical significant reduction in serum levels of TAC in RCS-exposed workers that can be attributed to the production of reactive oxygen species caused by the exposure to RCS in their workplaces. Free radicals produced can be eliminated by TAC and the decrease in the activity of this antioxidant show that exposure to crystalline silica can increase oxidative stress in body [33]. In line with our results, a previous study reported a reduction in TAC content in workers with direct exposure to cement [11]. In another study, it was showed a slight increase in levels of TAC among the exposed-workers compared to controls [17]. Unlike in other studies, it was reported that there was no significant difference in the level of TAC between the groups [34] .The conflicting findings mentioned in this study in levels TAC can be related to intensity of exposure, protective equipment, and nutritional status [17]. In general, there were some limitations in this study; for instance, the levels of RCS dusts did not measured in different parts of the workplace. Moreover, other enzymatic antioxidants such as superoxide dismutase, glutathione peroxidase and other non-enzymatic antioxidants (e.g. ascorbic acid and tocopherol) did not determined. However, of the evaluation of the studied parameters in several exposed workers in different work units with various exposure levels can be mentioned as strength of the study.

Conclusion

The present study showed that occupational exposure to RCS dusts can lead to damage to liver enzyme activity and oxidative stress as evidenced by increased serum levels of MDA, ROS and ALT and reduction in the level of TAC. To overcome these oxidative stresses, supplementation with dietary antioxidants, including ascorbic acid and A-tocopherol may suggestion beneficial health effects in the exposed workers. However, it is recommended to protect the health of workers

against silica dust they be use from industrial masks and proper ventilation system should be installed in the environment.

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Competing interests

All authors declared no conflict of interest regarding this paper.

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This study (Evaluation of Oxidative stress biomarkers and liver enzyme activity in workers occupationally exposed to respirable free crystalline silica) was reviewed and approved Research Ethics Committee of Hamadan University of Medical Sciences (IR.UMSHA.REC.1400.384).All volunteers provided informed consent prior to participation in this study.

Ethical considerations

Ethical issues (Including plagiarism, Informed Consent, misconduct, data fabrication and/or falsification, double publication and/or submission, redundancy, etc) have been completely ob-served by the authors.

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