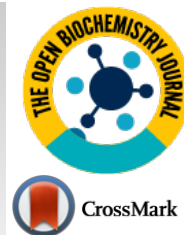




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## RESEARCH ARTICLE

### Investigation of Blood and Urine Malondialdehyde Levels in Mice Exposed to Silica Dust

Abdollah Gholami<sup>1</sup>, Farideh Golbabaei<sup>2,\*</sup>, Gholamheidar Teimori<sup>3,4</sup>, Mojtaba Kianmehr<sup>5</sup> and Mehdi Yaseri<sup>6</sup>

<sup>1</sup>Department of Occupational Health Engineering, School of Public Health, Birjand University of Medical Sciences, Birjand, Iran

<sup>2</sup>Department of Occupational Health Engineering, School of Public Health, Tehran University of Medical Sciences, Tehran, Iran

<sup>3</sup>Department of Environmental Health, School of Health, Torbat Heydariyeh University of Medical Sciences, Torbat Heydariyeh, Iran

<sup>4</sup>Health Sciences Research Center, School of Health, Torbat Heydariyeh University of Medical Sciences, Torbat Heydariyeh, Iran

<sup>5</sup>Department of Basic Sciences, Faculty of Medicine, Gonabad University of Medical Sciences, Gonabad, Iran

<sup>6</sup>Department of Biostatistics, School of Public Health, Tehran University of Medical Sciences, Tehran, Iran

#### Abstract:

#### Background and Objectives:

Occupational exposure to silica dust can lead to biochemical damage. Malondialdehyde (MDA) can be considered as a primary marker for measuring the level of oxidative stress in a living organism. This study was conducted in order to evaluate the level of MDA in blood and urine of mice exposed to silica dust.

#### Material and Methods:

In this experimental study, 72 mice (BALB/c) were randomly allocated to five exposed groups and 1 control group. Exposure of mice to pure 99% silica dust was done in closed containers. Blood sampling was performed from the heart of mice and urine sampling fulfilled by insertion into a metabolic cage. The RAO *et al.* method was used to measure MDA.

#### Results:

The highest level of plasma MDA in group 1 in the 4<sup>th</sup> month was 8.4±0.41 nmol/l and the lowest level of MDA was 1.3±0.2 nmol/l in the third sampling in the control group, also the highest amount of urine MDA in the first and second groups and 4 months after exposure was 1.16±0.51 nmol/l, and the lowest in the control group and in the third sampling was 0.48±0.06 nmol/l. A significant difference was found between the levels of MDA in all exposed groups at different times except for the 5<sup>th</sup> group with the lowest concentration ( $P < 0.05$ ).

#### Conclusion:

MDA in blood and urine could be proposed as a good biomarker for the evaluation of biochemical damages caused by silica dust. Measuring MDA is also a simple and inexpensive method that does not require complex equipment and can be used as an early detection test for biochemical damages caused by silica.

**Keywords:** Biomarker, Crystalline silica, Dust, Mouse, Malondialdehyde, Biochemical damages.

#### Article History

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## 1. INTRODUCTION

Silica is the most common mineral on earth and is one of the materials used in various mineral industries such as casting, glass processing, tile and ceramic factories, cleaners, supply of various chemicals and glazes, as well as in open and underground mines [1]. In such work environments, contact with air-

borne particles and dust is common and can cause many problems with regard to occupational and workers' health. Continuous inhalation of dust containing crystalline silica causes one of the most important pulmonary diseases called silicosis. In addition to silicosis, lung cancer, renal diseases and DNA damage (deoxyribonucleic acid), biochemical complications, and other complications have also been reported [2 - 5].

One of the damages caused by silica is biochemical damage that is studied by measuring biochemical indicators in the blood or urine. In previous field studies conducted on wor-

\* Address correspondence to this author at the Department of Occupational Health, School of Public Health, Tehran University of Medical Sciences, Iran; Tel: +9821-88951390; E-mail: [fgolbabaei@tums.ac.ir](mailto:fgolbabaei@tums.ac.ir)

kers exposed to crystalline silica, a significant difference was observed between the levels of Malondialdehyde (MDA) in workers exposed to silica compared to the control group [6 - 8].

No valid biomarker has been recommended for early diagnosis and measurement of the progression of silicosis so far [5, 9]. Aldehydes are produced from the oxidation of phospholipids in a cell membrane [10], and MDA is a secondary compound formed by the peroxidation of unsaturated fatty acids in the body that can be used as a marker to measure the level of oxidative stress in a living organism. On the other hand, since MDA is an active and highly reactive compound, with other attacking molecules, maintaining covalent bonding affects the function of the molecules and, finally, the cell's function [11 - 13].

The body cells are always exposed to various oxidizing substances. The Reactive Oxygen Species (ROS), which contain highly active oxygen molecules, are commonly used oxidizing compounds. These compounds attack different molecules, oxidize them, and produce secondary oxidation products. Lipids are the most important category of biomolecules that target reactive oxygen species. In fact, oxidative degradation of lipids by reactive oxygen species is called lipid peroxidation. Lipid peroxidation is usually performed on unsaturated fatty acids, and the final product is active aldehydes such as MDA [14 - 17]. Increasing levels of MDA and reducing the antioxidant defense system in the body can lead to cellular degradation [18]. The study of Khosrowbeigi *et al.* showed that lipid peroxidation of the membrane of the sperm increased the level of MDA and may be an important factor in reducing sperm motility [19]. According to previous studies, exposure to harmful noise in the workplace and/or smoking causes increased levels of MDA in individuals, which can increase the level of MDA in patients exposed to silica simultaneously [20, 21]. In the study by Zare Sakhvidi *et al.*, MDA level in exhale of workers exposed to silica dust was measured that did not differ significantly, but a positive and significant relationship was found between the intensity of exposure to dust and MDA in exhale of workers [22].

Determination of the amount of silica dust in various types of operations and mineral processes, and, also the influence of silica dust on genetic and biochemical damages is necessary. In this study, an animal study was used to remove the inevitable confounding factors present in the real space of the mines, and the studied mice were only exposed to silica dust. The results of this animal study could be used to validate the human study. Therefore, this study was conducted aimed to determine the level of MDA in blood and urine of the mice exposed to silica dust.

## 2. MATERIALS AND METHODS

In this experimental study, 72 mice (BALB/c) from the experimental center of Birjand University of Medical Sciences were evaluated to determine the effect of silica dust on the level of MDA in the mice blood and urine. The animals were kept in special animal shelves under standard dark conditions and free access to sufficient water and food at a temperature of

23±2°C. All experiments were performed in accordance with the international guidelines for working with experimental animals. The study was approved by the Ethics Committee of Tehran University of Medical Sciences and attempted to minimize the stress levels of the studied mice.

In this study, the mice were divided into 5 exposure groups and one control group. The mice in exposure groups were exposed to silica dust concentrations and the mice in the control group were not exposed to any chemical or other agents. The mice maintenance environment and diet were the same in all groups. The mice were exposed to silica dust in closed containers. The mice were exposed to silica for 8 hours a day, 6 days a week and 1, 2, 3 and 4 months, respectively. The silica used in this study was made of pure 99% silica, processed in Iran's mines, which is mostly used as casting sand and raw materials in tile and ceramic industries and glass factories.

### 2.1. Exposure to Silica Dust

The mice exposure containers with silica dust were 25×15×30 cm<sup>3</sup> seamless plastic containers fitted with 3 control valves, through a valve dust was inserted into the container from a uniform one, by another valve, the clean air was fed into the container and another valve was used for sampling and measuring dust inside the container by TSI device. The dust was created by a blower pump inside a container containing silica powder and introduced into a large glass container for first uniformity of dust, and then was guided through the hoses of the connector into the containers. Control valves were used to adjust dust concentration inside the containers. With measuring the amount of silica dust, the valve of the dust concentration in each container was controlled. Finally, the mean concentration during the exposure period was calculated. Fig. (1) shows a schematic representation of the system of the mice exposure to silica dust.

### 2.2. Measurement of MDA

In order to determine the level of MDA in mice blood and urine, blood sampling from the mice heart was performed and urine sampling fulfilled by insertion in a metabolic cage. The RAO *et al.* method was used to measure MDA. In this method, the amount of MDA is measured by Thiobarbituric method. For test, 100 µl of the serum sample was added to 900 µl of distilled water, then 500 µl of TBA agent was added to the diluted sample, placed at 100 °C for 1 h, after cooling the samples were centrifuged for 10 minutes at 4000 rpm and finally the supernatant is separated and optical absorbance of the samples is read by using a spectrophotometer at 534 nm wavelength. Using the silence factor (105×1.56), the concentration of MDA is calculated and the results are reported in micromoles per liter. Each experiment was repeated on each sample 3 times, and then the results were recorded as the mean.

### 2.3. Statistical Analysis

In order to study and analyze the data, software SPSS 21, one way ANOVA and Tukey post hoc tests were used. The significance level was less than 0.05.

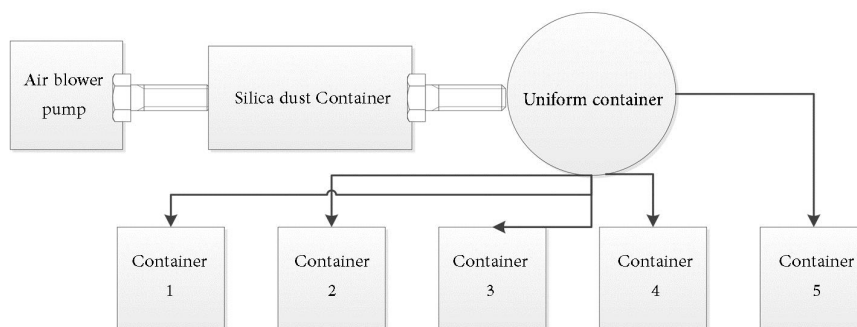


Fig (1). Schematic representation of the system of the mice exposure to silica dust.

### 3. RESULTS

The mean concentration and distribution of total dust size of exposure to silica in various containers are presented in Table 1. The highest concentration of exposure to dust in different groups was related to the container No.1. (Table 2) illustrates the mean of MDA (nmol/l) in blood plasma of the mice exposed to dust. The highest level of MDA in group 1 in the 4th month was  $8.4 \pm 0.41$  nmol/l and the lowest level of MDA in the third sampling in the control group was  $1.3 \pm 0.2$  nmol/l. Using ANOVA test, a significant difference was observed between the levels of blood plasma MDA in all

groups exposed at different times except group 5 with the lowest concentration ( $P < 0.05$ ).

The mean urine MDA (nmol/l) of mice exposed to dust is shown in Table 3. The highest level of urine MDA in groups 1 and 2 and the fourth month after exposure was  $1.16 \pm 0.51$  nmol/l and the lowest level of MDA in the control group and in the third sampling was  $0.48 \pm 0.06$  nmol/l. The results of ANOVA statistical test showed a significant difference between the levels of urine MDA in all the different groups exposed at different times except for the group 5 with the lowest concentration ( $P < 0.05$ ).

Table 1. The mean concentration and distribution of silica dust in different containers of exposure.

Container	Dust concentration (mg/m <sup>3</sup> )				
	PM <sub>1</sub>	PM <sub>2.5</sub>	PM <sub>5</sub>	PM <sub>10</sub>	Total
1	6.8	7.8	11.7	15.5	17
2	4.9	5.56	8.32	10.5	12
3	3.1	3.7	5.74	6.59	8
4	1.42	1.9	2.013	2.7	3
5	0.82	1.01	1.22	1.23	1.3
6(Control)	0	0	0	0	0

Table 2. The mean malondialdehyde (n mol/l) of blood plasma of mice exposed to dust.

Exposure time	Group 1	Group 2	Group 3	Group 4	Group 5	Control
1 month	$3.8 \pm 0.52$	$5.2 \pm 2.5$	$4.06 \pm 2.7$	$3.2 \pm 1$	$1.53 \pm 0.66$	$4.3 \pm 2.8$
2 month	$5.1 \pm 0.152$	$4.7 \pm 0.95$	$3.8 \pm 0.66$	$4.1 \pm 0.57$	$3.23 \pm 0.57$	$1.5 \pm 0.47$
3 month	$7.3 \pm 0.57$	$6.1 \pm 0.78$	$5.5 \pm 1.09$	$5.9 \pm 0.85$	$4.3 \pm 1.1$	$1.3 \pm 0.2$
4 month	$8.4 \pm 0.41$	$6.8 \pm 0.57$	$6.6 \pm 0.43$	$6.6 \pm 0.38$	$6.1 \pm 0.57$	$1.8 \pm 0.58$
p-value	0.001	0.001	0.003	0.001	0.26	-

Table 3. The mean malondialdehyde (n mol/l) of urine of mice exposed to dust.

Exposure time	Group 1	Group 2	Group 3	Group 4	Group 5	Control
1 month	$0.97 \pm 0.0057$	$0.15 \pm 0.159$	$0.84 \pm 0.44$	$0.803 \pm 0.37$	$0.1 \pm 0.054$	$0.55 \pm 0.1$
2 month	$1.09 \pm 0.11$	$0.87 \pm 0.011$	$0.98 \pm 0.22$	$0.88 \pm 0.1$	$0.67 \pm 0.11$	$0.55 \pm 0.1$
3 month	$1.2 \pm 0.15$	$1.02 \pm 0.06$	$1.02 \pm 0.21$	$0.712 \pm 0.56$	$0.803 \pm 0.21$	$0.48 \pm 0.6$
4 month	$1.16 \pm 0.51$	$1.16 \pm 0.57$	$1.16 \pm 0.72$	$0.02 \pm 0.06$	$0.73 \pm 0.21$	$0.51 \pm 0.12$
p-value	0.0001	0.007	0.001	0.023	0.704	-

#### 4. DISCUSSION

In order to evaluate oxidative stress, measurement of MDA produced in the peroxidation lipid process is used [23]. The amount of MDA produced is proportional to the failure and separation of unsaturated fatty acids. Therefore, the measurement of MDA in various biological samples is a good index for lipid peroxidation and can be used as a marker for measuring oxidative stress levels in a living organism [24].

According to conducted studies, one of the biochemical studies of exposure to silica has been suggested through a review of blood and urine MDA [23, 25]. Hence, in the present animal study, the level of MDA in blood and urine of the mice exposed to silica dust was studied. The study results showed that with increasing the level of silica dust and exposure time, the level of MDA in blood and urine in mice increased, so that in groups 5 and 4 most exposed for a long time the highest level of MDA was found in their blood and urine, and in all groups, the level of MDA in blood and urine was significantly different from that of the control group. In a study by Azari *et al.*, which was conducted on workers exposed to silica in sandblasting, the concentration of urine MDA and neoprene in workers exposed to silica was higher than that of the control group [8].

In a study by Kamal *et al.* on workers exposed to silica in Egypt, serum MDA concentration was higher in workers exposed to silica, which is consistent with the results of the present study [7]. In a study by Jafari *et al.* on workers exposed to silica in sandblasting, the level of MDA and neoprene urine in workers exposed to silica was higher than that of the control group [26]. In a study by Orman *et al.* on cement workers in Turkey, MDA plasma was significantly increased in workers exposed to silica compared to the control group [6] which is consistent with the results of the present study. The difference in MDA levels in the experimental and control groups indicates lipid oxidation in the mice exposed to silica dust, indicating that MDA can be used as a biomarker for workers exposed to silica dust.

A significant difference was observed between the levels of MDA in urine and blood in all groups at different times, except for the 5th group with the lowest concentration, indicating that the amount of silica dust and exposure time can cause increasing MDA level in the mice.

Given that in several human studies a significant difference was observed between the levels of MDA in groups exposed to silica dust and the control group, it seems necessary to conduct a study with more samples in a longer period of time.

#### CONCLUSION

The study results showed that MDA in blood and urine could be an appropriate biomarker for the evaluation of silica dust damages. Measuring MDA is a simple and inexpensive method that does not require complex equipment and can be used as an early detection test for biochemical damages caused by silica.

#### ETHICS APPROVAL AND CONSENT TO PARTICIPATE

This study was approved by Birjand University of Medical Sciences, Birjand, Iran.

#### HUMAN AND ANIMAL RIGHTS

No humans were involved in this study. All procedures were performed in accordance with the standards of The US Public Health Service's "Policy on Humane Care and Use of Laboratory Animals," and "Guide for the Care and Use of Laboratory Animals."

#### CONSENT FOR PUBLICATION

Not applicable.

#### FUNDING

None.

#### CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

#### ACKNOWLEDGMENTS

Declared none.

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