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EXHALED BREATH MALONDIALDEHYDE, SPIROMETRIC RESULTS AND DUST EXPOSURE ASSESSMENT IN CERAMICS PRODUCTION WORKERS

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Abstract
Objectives: The study aimed at measuring exhaled breath malondialdehyde (EBC-MDA) in workers exposed to dust containing silica and at its comparison with the non-exposed control group. **Material and Methods:** The cross sectional, case-control study (N = 50) was performed in a tile and ceramics production factory in Yazd, Iran. EBC-MDA was quantified in exhaled breath of the participants by a lab made breath sampler. Exposure intensity was measured according to the NIOSH 0600 method in selected homogeneous exposure groups. Additionally, spirometry test was conducted to investigate a correlation between EBC-MDA and spirometric findings in the exposed workers. **Results:** There was no difference in the observed exposure intensities of silica containing dust in different units. However, "coating preparation" was the unit with the highest concentration of dust. Although, the level of EBC-MDA in the cases was slightly higher than in the controls, the difference was not statistically significant (U = 252, p = 0.464). A significant and positive correlation was found between dust exposure intensity in working units and the measured EBC-MDA of workers (r = 0.467, N = 25, p = 0.027). There were also no statistically significant differences among job categories in the exposed group for the values of FEV_1% (F(3, 44) = 0.656, p = 0.584), FVC% (F(3, 44) = 1.417, p = 0.172), and FEV_1/FVC% (F(3, 44) = 1.929, p = 0.139). **Conclusions:** The results showed a significant correlation between respirable dust exposure intensity and the level of EBC-MDA of the exposed subjects. However, our results did not show a significant correlation between lung function decreases and EBC-MDA.

Key words:
Oxidative stress, Occupational exposures, Spirometry, Breath condensate, Malondialdehyde

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INTRODUCTION

Silica is the 2nd most abundant mineral in the earth crust. This compound is used as a main raw material in the production of ceramics. Therefore, occupational exposure to silica occurs at different stages in ceramic manufacturing [1]. Prolonged exposure to crystalline silica may lead to serious lung diseases such as silicosis [2,3]. Recent findings suggest that silica particles that reach lung parenchyma can be phagocytized by lung macrophages, and thus, activate generation of reactive oxygen species (ROSs) and inflammatory cytokines [4]. Imbalance between the free radicals and ROSs and antioxidant reserve may result in oxidative stress [5]. Investigation of per-oxidation products is a suitable approach when one aims at determination of the presence and also of the degree of lesions due to oxidative stress in subjects exposed to dusts containing silica.

Until now no valid biomarker has been recommended for early diagnosis and measurement of silicosis' progress [6,7]. Aldehydes, which are known as biomarkers of oxidant-induced damage, are generated from oxidation of phospholipids in a cell membrane [8]. Malondialdehyde (MDA), one of the common secondary products of lipid per-oxidation, is used for quantification of oxidative stress in human body [9]. The silica-induced ROSs lead to an increase in MDA levels and consequently to depletion of antioxidant capacity [10,11]. There are several studies in which plasma MDA has been used as a marker of oxidative stress for dust exposure scenarios [11,12]. Exhaled breath condensate (EBC) has been proposed as an interesting alternative matrix of biomarkers in the prognosis and diagnosis of lung diseases [13,14]. Breath matrix has some advantages such as readiness to use, non-invasiveness and accessibility. Recently, several studies have been performed on the applicability of biomarkers of EBC in both occupational and environmental exposure assessments [14–16]. To the best of our knowledge, there is no similar study dealing with the level of MDA in the exhaled breath condensate (exhaled breath malondialdehyde – EBC-MDA) of the workers exposed to silica containing dusts.

In the present study, the objective was to measure the level of EBC-MDA of the workers exposed to dust containing silica and to compare its intensity with the level of MDA in a non-exposed control group. Spirometry test was also conducted to investigate the correlation between exhaled breath MDA and spirometric findings in the exposed workers. We hypothesized that the level of EBC-MDA in the silica exposed workers would show a significant correlation with their exposure intensity.

MATERIAL AND METHODS

Study and control group

The current study was a cross sectional, case-control study that was performed in a ceramics production factory in Yazd, Iran from April to July 2013. The exposed group consisted of 25 male workers with duration of employment longer than 2 years from different units of the factory. The control group consisted of 25 male subjects from administrative departments of the same factory without occupational exposure to dust. In order to minimize the effect of possible confounding variables, the subjects with the history of asthma, respiratory disease in the past 12 months, chronic respiratory and lung disease were excluded from the study. The subjects were asked to disclose their job history, socio-demographic characteristics, smoking habits and medical history by filling in a questionnaire. Medical files of all the participants were checked ensuring their past smoking status so that smokers as well as ex-smokers were excluded from the study. All of the subjects were informed about the purpose of the study. An informed consent was obtained from all the participants. The study protocol was in accordance with the Helsinki Declaration and was approved by the ethics committee of Shahid Sadoughi University of Medical Sciences, Yazd, Iran.

Spirometry test

All spirometry measurements were performed using an autocalibrated flow-type spirometer (Spirolab III, Mir, Italy) according to the guidelines of the American Thoracic Society/European Respiratory Society (ATS/ERS) [17–20]. Age of the participants was obtained by means of self-report, while their weight, without shoes, was measured using a digital scale (Laica, Italy). All the tests were performed in a sitting position in the morning. Room temperature was kept between 20 and 26°C. Spirometry results were automatically corrected for body temperature pressure saturation (BTPS) conditions by the software of the device. Acceptability criteria were considered according to the ATS/ERS taskforce [17,19]. The highest sum of the forced vital capacity (FVC) and forced expiratory volume in 1 s (FEV_1) were selected from 3 technically acceptable recordings. Before the test, all factors intervening or contraindicating the spirometry were questioned [17].

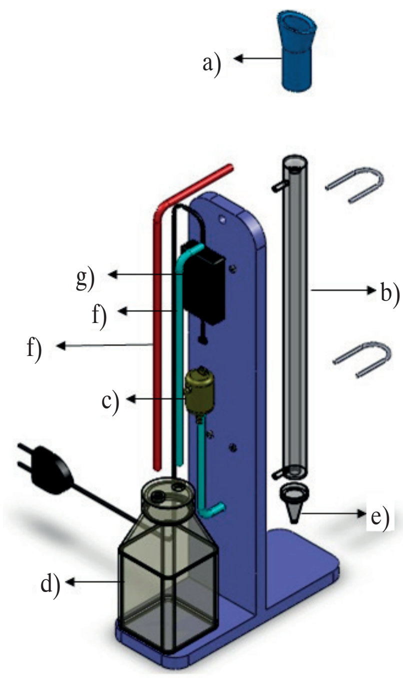

Fig. 1. Exploded view of a breath sampler: a) mouthpiece, b) condenser, c) water pump, d) ice-water reservoir, e) sample collector, f) water circulation tubes, g) digital thermometer

Breath sampling

A laboratory made breath sampler was used to collect EBC. The sampler consisted of a glass tube condenser that was filled with chilled water (maintained at 0°C). The water was circulated by an electric pump (12 V windshield wipers water pump) and was constantly mixed with ice-water solution to maintain the condenser temperature at 0°C. The temperature was continuously monitored using an electronic thermometer. To collect the EBC, the participants were asked to blow into a mouthpiece attached to the condenser. During breath sampling, the subjects wore a nose clip to breathe tidally through a mouthpiece. All the participants were trained to breathe at normal frequency for a period of 5 min. Condensates were formed on the inner surface of the device and were collected in a microtube attached to the end side of the device. Exhaled breath condensate samples were immediately stored in a –20°C freezer until preparation for high performance liquid chromatography (HPLC) analysis. After each sampling, and before the next sampling sequence, the condenser was gently washed and rinsed with normal saline and then dried by a hair drier. Operating parameters of the sampler were optimized and published elsewhere [21]. Figure 1 shows the exploded schematic diagram of the breath sampler.

Sample preparation and analysis

Exhaled breath malondialdehyde was analyzed by an HPLC equipped with a fluorescent detector (Waters 515 HPLC, detector: fluorescence Waters 474) according to the method developed by Larstad et al. [22]. Briefly, 50 μl of EBC was added to 2-thiobarbituric acid (TBA) (Merck, Germany), vortex-mixed for 1 s, and then it was placed in a 95°C water bath for 60 min for derivatisation to take place. After cooling, the sample was analyzed by a reversed-phase HPLC with a C18 column using methanol (HPLC grade, Merck, Germany) and acetonitrile

(HPLC grade, Merck, Germany) as a mobile phase [22]. All peaks were quantified using Empower pro chromatographic software. The wavelength of the florescent detector was set at 532 nm for excitation and at 553 nm for emission, with 100 gain.

Inhalation exposure assessment

Inhalation exposure was assessed based on similar exposure group (SEG) strategy in all units of the factory. Due to the similarity of tasks within a unit and also the homogenous dispersion of pollutants, all the workers of each production unit were categorized as a SEG. Accordingly, 9 air samples were collected from the breathing zone of the selected workers of 3 predefined SEGs. Air sampling was carried out according to the National Institute for Occupational Safety and Health (NIOSH) manual for analytical method 0600 [23]. The respirable fraction of dust was captured on fiberglass filters using a cyclone connected to a personal sampling pump for gravimetric analysis. The flow rate of the pump was adjusted to 1.7 l/min during the sampling time (at least 30 min). The result of gravimetric analysis of the collected respirable fraction of dust was used as a surrogate for crystalline silica exposure intensity.

Statistical analysis

Data were analyzed using statistical package for social sciences (SPSS) (SPSS, Chicago, Illinois, USA). The distribution of parameters was examined with Kolmogorov-Smirnov test. The mean values of spirometric parameters were compared by means of the Student's t test between the exposed and control groups. Nonparametric Mann-Whitney U test was used to examine the mean of MDA levels between the exposed and control groups. One-way ANOVA was used to test the mean of EBC-MDA concentration among different job groups. ANOVA test was performed on log transformed values of EBC-MDA. The Pearson's r coefficient was computed to assess the relationship between EBC-MDA and exposure intensity. In all the tests, the level of significance was set at $p < 0.05$.

RESULTS

Study groups

Table 1 shows demographic characteristics of the subjects including age, years of exposure, height, and weight. The mean age of the exposed and control groups were 33.84 years (range: 23–47) and 33.6 years (range: 24–43), respectively. The controls were matched to the exposed subjects in terms of age, height and weight ($p < 0.05$).

Monitoring data

Totally, 9 air samples were collected from 3 units of the factory according to the NIOSH 0600 method (Table 2). All the samples were above the limit of detection (LOD).

Table 1. Demographic characteristics of the control and exposed groups

Parameter	Group (M±SD)		Test result	
	control (N = 25)	exposed (N = 25)	t	p
Age (years)	33.60±6.12	33.84±6.67	0.132	0.890
Duration of employment (years)	10.72±5.27	8.20±4.54	–1.810	0.076
Height (cm)	172.00±5.00	172.00±6.00	0.432	0.670
Weight (kg)	75.96±16.38	80.60±13.16	1.090	0.280

M – mean; SD – standard deviation.

Table 2. Inhalation exposure intensity in various units of the factory

Exposed group	Respondents (n)	Exposure (mg/m^3)			
		M	SD	min.	max
Ball mill	3	8.00	0.360	7.7	8.4
Press	3	4.80	0.655	4.1	5.4
Coating preparation	3	11.96	0.802	11.2	12.8

min. – minimal value; max – maximal value.
Other abbreviations as in Table 1.

The intensity of exposure to dust containing silica was not significantly different among 3 units. However, in "coating preparation" unit, the concentration of dust was noticeably higher than in the 2 other units.

Spirometric results

Spirometry tests were performed for 50 male workers during a period of 12 weeks, from April to July 2013 (Table 3). The mean value of FEV_1/FVC% was 86% (SD = 15.3%) and 83.26% (SD = 5.70%) for the exposed and control groups, respectively. The mean value of FVC% was obtained and it amounted to 92.72% (SD = 10.96%) and 98.22% (SD = 8.88%) for the exposed and control groups, respectively. No statistically significant differences were observed for the mean of FEV_1/FVC% and FVC% between the exposed and control groups ($p = 0.422$ and 0.064, respectively). Also no significant differences were observed for the mean of FEV_1% ($F(3, 44) = 0.656$, $p = 0.584$), FVC% ($F(3, 44) = 1.417$, $p = 0.172$) and FEV_1/FVC% ($F(3, 44) = 1.929$, $p = 0.139$) among the jobs of the exposed group. All of the participants had normal spirometric parameters, meaning the absence of either restrictive or obstructive lung diseases.

MDA measurements

The level of MDA in EBC of all the subjects is shown in Table 4. It is worth mentioning that the concentration of EBC-MDA was not normally distributed ($p = 0.043$). Although the level of EBC-MDA in the cases was slightly higher than in the controls, its mean value was not statistically different between the 2 groups ($U = 252$, $p = 0.464$). The result of ANOVA test on

Table 3. Pulmonary function test data of the controls and exposed workers

Group	Test (%) (M±SD)		
	FEV_1	FVC	FEV_1/FVC
Exposed (N = 25)	92.52±12.19	92.72±10.96	86.00±15.31
Press (N = 8)	93.13±6.06	91.88±4.05	93.41±24.55
Ball mill (N = 13)	91.77±15.55	94.23±13.92	82.06±7.79
Coating preparation (N = 4)	93.75±11.38	89.50±11.09	84.01±2.91
Control (N = 25)	96.87±9.26	98.22±8.88	83.26±5.70

FEV_1 – forced expiratory volume in 1 s; FVC – forced vital capacity. Other abbreviations as in Table 1.

Table 4. Exhaled breath malondialdehyde (EBC-MDA) in the exposed and control groups

Group	Respondents (n)	EBC-MDA (nM)			
		M	SD	min.	max
Exposed (total)	25	4.58	0.48	4.11	6.32
Exposed (press)	8	4.37	0.16	4.20	4.66
Exposed (ball mill)	13	4.55	0.37	4.11	5.35
Exposed (coating preparation)	4	5.10	0.88	4.32	6.32
Control	25	4.44	0.61	3.52	5.55

Abbreviations as in Tables 1 and 2.

log transformed concentration of EBC-MDA showed no significant difference in the mean value among 4 job categories, $F(3, 44) = 1.8$, $p = 0.161$.

Relationship between EBC-MDA level and spirometric findings

We found a significant negative correlation between the level of EBC-MDA and working history ($r = -0.283$, $N = 50$, $p = 0.047$). However, a negative but insignificant correlation was observed between the level of EBC-MDA and decline in spirometric parameters. A significant and positive correlation was found between dust exposure intensity and the level of EBC-MDA ($r = 0.467$, $N = 25$, $p = 0.027$).

DISCUSSION

In this study, we measured lung function, the level of EBC-MDA and exposure intensity for a group of workers in a ceramics production factory and the results were compared with a matched control group. The results showed a significant correlation between the respirable dust exposure intensity (as the representative of silica exposure) and the level of EBC-MDA of the exposed subjects. Romieu et al. [24], in the study on asthmatic children exposed to air pollution, have also found that exposure to air pollution is related to the increase in EBC-MDA level. It has revealed that 2.5 mg increase in air pollution level leads to 1.12 nM EBC-MDA.

In our study a significant correlation was not established between the decreased lung function and the increased EBC-MDA, while Romieu et al. [24] have reported an inverse correlation for EBC-MDA with FVC and FEV_1. Orman et al. have observed a negative correlation between plasma MDA and FEV_1 and also between plasma MDA and $FEV_1\%$ [11]. Their findings suggest that the increased lipid peroxidation was associated with pulmonary airway narrowing in the general population [25].

Malondialdehyde levels in both silica and asbestos exposed groups were significantly higher than in their controls. Also the level of MDA in asbestos exposed workers was significantly higher than in the case of the silica exposed ones [26]. Short employment duration of the exposed subjects in the current study may be the best reason for the obtained results that are in favor of null hypothesis of no difference in EBC-MDA between the cases and controls. Exposure to environmental and outdoor air pollution, due to fugitive emissions, could be considered as a source of exposure for the subjects that are normally classified as controls. This condition may increase the level of MDA and oxidative stress and hence, can act as a confounding parameter, leading to insignificant difference in EBC-MDA of the cases and controls. However, Aydin et al. have reported a higher level of plasma MDA in cement-exposed workers [27]. In another study, silicosis was associated with the increased plasma MDA and reduced erythrocyte glutathione levels, providing an oxidative link [11].

Because of the lack of a standard protocol across the studies of breath sampling, our results cannot be compared with other findings in terms of net amount of EBC-MDA. Several studies have reported EBC-MDA levels (nM) for various operations. However, there is a large variation across the studies [24]. The obtained values of EBC-MDA in our study were close to the amounts reported by Larstad et al. [22].

There are several limitations of our study. First, we used a lab-made system for collection of EBC. Therefore, our results are not comparable with other studies. Second, we did not use a saliva trap in EBC collection system. Therefore, it is possible that in our study saliva could act as a confounding factor. We used a respirable fraction of total dust exposure as a surrogate of silica exposure. However, as a part of occupational health program by regulatory bodies in the province, the presence of silica in air samples of ceramic industries was confirmed. These findings are used across the province as a rule of thumb for estimation of silica exposure from gravimetrically acquired dust samples. That is why, we used the total respirable dust as a surrogate of silica exposure. However, in future studies of exposure assessment it would be better to use more accurate silica determination methods such as X-ray diffraction.

In this study, the controls had longer employment duration than the cases, which may be another reason for disagreement of our findings with majority of the other studies. We excluded the cases with the history of cigarette smoking (current smokers and ex-smokers), but based on our past experiences in the field, the workers' self-reported tobacco use could be suspicious and inaccurate. We used a standard case-control design with strict inclusion and exclusion criteria, but it would be also interesting to include positive controls in the study. Unfortunately, we had several limitations concerning the access to positive controls. However, in future studies we suggest the use of positive controls.

CONCLUSIONS

In conclusion, we found that EBC-MDA concentration in the exposed subjects is related to their exposure intensity. Therefore, it may be valuable to use EBC-MDA as a non invasive biomarker of exposure to silica containing dust in ceramics production and other similar industries. However, keeping in mind the limitations mentioned above, our results should be used with caution. Further research is necessary to validate this biomarker as a reliable biomarker for occupational exposure assessment studies.

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