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Assessment of DNA damage in ceramic workers

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Abstract

It is known that ceramic workers are potentially exposed to complex mixture of chemicals such as silica, inorganic lead, lime, beryllium and aluminum that can be associated with an increased risk of several diseases. All operations in the ceramic industries such as mixing, moulding, casting, shaking out and finishing jobs, have been associated with the higher exposure levels and in most of the silica-related industries, average overall exposure exceeded permissible exposure levels for respirable crystalline silica. The aim of this study was to evaluate the possible genotoxic damage in ceramic workers exposed to complex mixture of chemicals mainly crystalline silica. For this purpose, the blood and buccal epithelial cell samples were taken from the ceramic workers ($n = 99$) and their controls ($n = 81$). The genotoxicity was assessed by the alkaline comet assay in isolated lymphocytes and whole blood. Micronucleus (MN), binucleated (BN), pyknotic (PYC), condensed chromatin (CC), karyolytic (KYL), karyorrhectic (KHC) and nuclear bud (NBUD) frequencies in buccal epithelial cells and plasma 8-oxo-7,8-dihydro-2′-deoxyguanosine (8-oxodG) levels were also evaluated. In the study, 38 workers were diagnosed with silicosis, 9 workers were suspected to have silicosis, whereas 52 workers were found to be healthy. DNA damage in blood and lymphocytes; MN, CC + KHC, PYC frequencies in buccal epithelial cells and 8-oxodG levels in plasma were increased in workers compared to their controls. These results showed that occupational chemical mixture exposure in ceramic industry may cause genotoxic damage that can lead to important health problems in the workers.

Introduction

All operations in the ceramic industries such as mixing, moulding, casting, shaking out and finishing jobs, have been associated with the higher exposure levels and in most of the silica-related industries, average overall exposure exceeded permissible exposure levels for respirable crystalline silica (1). Health risks of occupational silica-containing dust exposure have been evaluated in coal miners, foundry, pottery, granite foundry and cement plant workers, sandblasters and stone crushers. Occupational exposure to silica dust in workers is still considered to be an important health problem especially in developing countries (2–8). It is estimated that at least two to three million of workers worldwide are occupationally exposed to silica annually and inhalation of silica can lead to silicosis and cancer (9,10).

Crystalline silica was recently re-evaluated by the International Agency for Research on Cancer (IARC) and classified as a human carcinogen (Group 1). In the overall evaluation, however, it was noted that carcinogenicity was not detected in all industrial circumstances and that it may depend on the characteristics of silica or on external factors. The mechanisms of crystalline silica-induced carcinogenesis are so far only partially understood. *In vivo* and *in vitro* studies showed that an inflammation dependent mechanism involving inflammatory cell derived reactive-oxidant species (ROS) and growth stimulatory factors is an important mechanism of action (11). However, on

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the basis of some data, an additional role of silica surface derived ROS and of direct genotoxic effects of particles can be considered (12,13).

This study aimed to investigate the possible genotoxic effects associated with crystalline silica-rich dust exposure in Turkish ceramic workers using the single cell electrophoresis (comet) and the micronucleus (MN) assays. The comet assay has been found to be a very sensitive, rapid, reliable and fairly inexpensive way of measuring DNA damage. It has a further advantage that the observations are made at the single cell level. Moreover, it is an invaluable tool for investigating DNA damage in human populations (14). To substantiate our results and to provide a cytogenetic parameter, the MN test was also carried out in buccal epithelial cells. Since epithelial cells can be obtained easily by relatively non-invasive methods and indicate toxicity in actual target tissue by the MN assay, their usage in case–control studies have been increasing (15–18). This test also allows the detection of both clastogenic and aneugenic agents (19). In addition to these, plasma 8-oxo-7,8-dihydro-2′-deoxyguanosine levels (8-oxodG) were also evaluated. The influence of confounding factors like age, smoking, alcohol drinking, duration of exposure on the differences in DNA damage, buccal MN frequencies and plasma 8-oxodG levels were also analyzed. The measurements related to workplace respirable dust levels, chest X-ray films and spirometry tests of subjects were also evaluated.

Materials and methods

This study was approved by Hacettepe University Clinical Research Ethical Committee (Date and Number: 20/11/2014, KA-14038).

Subject selection

The study group consisted of 99 male workers from a Turkish Ceramic Plant exposed to crystalline silica-containing dust. Male office workers (n = 81) without dust exposure comparable for age and smoking habits to the workers were selected as the control group. In order to mitigate the influences of genetic and factors related to lifestyle habits, all male participants were selected from same region. Each participant completed a detailed questionnaire which included questions regarding working conditions, possible confounding factors such as smoking, alcohol consumption and nutritional habits. Written informed consent was obtained from the donors. All samples have been taken in the Ankara Occupational Diseases Hospital from June 2014 to March 2016.

Biological sampling

Approximately 4 ml whole blood samples were taken by a stainless steel needle from the left arm cubital vein in tubes with anti-coagulant (heparin). 500 µl whole blood was centrifuged immediately at 800 g for 15 min to obtain plasma for the determination of 8-oxodG levels and the samples were aliquoted and kept at –80°C until analysis.

Three milliliters whole blood was mixed with 3 ml ice cold phosphate-buffered saline (PBS) and lymphocytes were then isolated by Ficoll–Hypaque density gradient procedure for comet assay (20). 500 µl whole blood was also used for the the assay. Cells were checked for viability by trypan blue exclusion. Cell concentrations were adjusted to ~2 × 10^5/ml in the buffer and the assay was performed in the same day after biological sampling.

Subjects were asked to rinse their mouths with water and buccal cell samples were obtained by gently rubbing the inside of the cheeks (right and left side) with a wet tongue depressor. The collected cells were smeared directly onto wet slides and then left to dry.

Samples from both workers and controls were analysed on the same day of sampling for the comet and MN assays.

Chemicals, kit and equipment

The chemicals used in these experiments were purchased from the following suppliers: normal melting agarose (NMA) and low melting agarose (LMA) from Boehringer (Mannheim, Germany); sodium chloride (NaCl), sodium hydroxide (NaOH), activated coal, methanol, ethanol, HCl, sodiumbisulphite, pararosaniline, fast green and xylene from Merck Chemicals (Darmstadt, Germany); dimethylsulfoxide (DMSO), ethidium bromide (EtBr), Triton X-100 and PBS tablets from Sigma (St. Louis, MO); ethylenediamine tetraacetic acid disodium salt dihydrate (EDTA-Na_2), natriumlauroylsarcosinate and tris(hydroxymethyl) aminomethane from ICN Biomedicals Inc. (Aurora, OH, USA); 8-oxodG kit was obtained from Cayman Chemical Company (Ann Arbor, MI, USA). For spectrophotometric measurement, SpectraMax M2 (Molecular Devices, Sunnyvale, CA, USA) and for quantification SoftMax Pro Software (Molecular Devices, Sunnyvale, CA, USA) were used.

Radiological examination and spirometry test

Posteroanterior chest (PA) radiographs of exposed workers were taken in the radiology clinic of Ankara Occupational Diseases Hospital. A short exposure time with high voltage technique was used (Trophy UFXRAY, 500 mA, TM). The radiographs were read by a specialist according to the International Labour Office classifications (21).

Standard spirometry measurements of workers and controls were done with a dry-seal spirometry (Zan 100, nSpire Health Inc., Oberthulba, Germany). Pulmonary function tests were interpreted in accordance with the American Thoracic Society standards (22).

Exposure measurements

Collection and measurement of the dust samples were performed by a nationally accredited laboratory. Dust samples were collected from the level of respiration on the daily shift (8 h) of workers with badge dosimeter (Buck, Libra Plus-5). The pore size of cellulose membrane filters was 0.8 µm. Respirable total dust concentrations in mg/m^3 were measured by the gravimetric analyses according to the Health and Safety Executive method MDHS 14/3.

Single cell gel electophoresis (Comet) assay

The basic alkaline comet assay of Singh *et al.* (23) as further described by Tice *et al.* (24) was performed. The isolated lymphocytes and leukocytes were embedded on agarose gel, lysed and fragmented DNA strands drawn out by electrophoresis to form a comet. After electrophoresis, the slides were neutralized and then incubated in 50, 75 and 98% of alcohol for 5 min each. The dried microscopic slides were stained with EtBr (20 mg/ml in distilled water, 60 ml/slide) and scored with a Leica® (Germany) fluorescence microscope under green light. The microscope was connected to a charge-coupled device camera and a personal computer-based analysis system (Comet Analysis Software, version 3.0, Kinetic Imaging Ltd, Liverpool, UK) to determine the extent of DNA damage after electrophoretic migration of the DNA fragments in the agarose gel. In order to visualize DNA damage, 100 nuclei per slide were examined at 40× magnification. Results were expressed as the percent of DNA in tail (tail intensity).

Buccal MN cytome assay (BMCyt assay)

Within the same day of sample collection, the slides were transferred to the laboratory and fixed in 80% methanol and air-dried. Slides

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were treated in 1 N HCl for 2 min at room temperature, 10 min at 60°C and 2 min at room temperature, respectively. After washing in distilled water, the slides were left to dry. Feulgen staining (1 g pararosaniline in 100 ml distilled water) was then carried on at room temperature in the dark for 90 min and followed by 5 min washing in distilled water. The slides were then stained in fast green solution (0.5 g fast green in 95% ethanol) for 10 s, kept in xylene for 10 min and finally air-dried. The BMCyt assay has been used to measure biomarkers of DNA damage (micronuclei and/or elimination of nuclear material by budding buds), cytokinetic defects (binucleated cells) and proliferative potential (basal cell frequency) and/or cell death [condensed chromatin (CC), karyorrhectic (KHC), pyknotic (PYC) and karyolitic (KHC) cells]. For each volunteer, 2000 buccal cells (1000 from each of the duplicate slides) were scored by light microscopy as suggested by Sarto *et al*. (25) and Thomas *et al*. (26) and cells frequencies were expressed as per thousand.

Plasma 8-hydroxydeoxyguanosine (8-oxodG) levels

Plasma 8-oxodG levels were determined by using kit following the manufacturer's procedures at a wavelength between 405 and 420 nm. The results were expressed as pg/ml.

Statistical analysis

Analysis of data was performed using the computer program SPSS 20.0 for Windows. The distribution of the data was checked for normality using the Kolmogorov–Smirnov test. The homogeneity of the variance was verified by the Levene test. Data are expressed as mean ± SD for continuous variables and the number of cases per cent (%) for categorical variables. The differences among the groups with normal distribution were evaluated by Student's *t* test for two independent groups and one way analysis of variance (ANOVA) for more than two groups. Post hoc analysis of group differences was performed by the least significant difference (LSD) test. The differences among the groups without normal distribution were evaluated by Mann–Whitney *U* for two groups, Kruskal–Wallis test for more than two groups. The magnitude of linear relationship was calculated by Pearson correlation analysis. To determine the most effective parameters on the results, multiple linear regression analysis was used. Values of $P < 0.05$ were considered as statistically significant.

Results

General characterization of the study subjects

The general characteristics of the study subjects are shown in Table 1. Age, smoking and alcohol habits were comparable between the groups. The safety equipments were claimed to be used by most of the workers. 69.7, 61.6 and 34.3% of the workers used masks gloves and safety google, respectively. However, it was observed that the necessary equipments were not suitable for a better protection as masks were made from permeable fabrics. The average cigarette consumption of smoking workers and controls was nearly 20 cigarettes per day.

Radiological examination and spirometry test

Thirty-eight workers were diagnosed with silicosis, 9 workers were suspected to have silicosis, whereas 52 workers were normal. According to the ILO classification, PA chest radiographs of 32, 4 and 2 silicotic workers were found to be categories 1, 2 and 3, respectively.

Table 1. Demographic characteristics of the study population

Factors	Controls (n = 81)	Workers (n = 99)
Age (years)[a]	44.7 ± 9.6	35.5 ± 5.8
18–29	11 (13.6%)	16 (16.1%)
30–42	12 (14.8%)	75 (75.8%)
>42	58 (71.6%)	8 (8.1%)
Smokers	48 (59.3%)	33 (33.3%)
Non-smokers	33 (40.7%)	66 (66.7%)
Alcohol consumption		
No	81 (100%)	83 (83.8%)
Yes	0 (0%)	16 (16.2%)
Duration of exposure (years)		
0–5		21 (21.2%)
6–10		46 (46.5%)
11–15		19 (19.2%)
16–20		13 (13.1%)
Using protective equipment		
Gloves		
No		17 (17.2%)
Sometimes		21 (21.2%)
Yes		61 (61.6%)
Mask		
No		30 (30.3%)
Yes		69 (69.7%)
Safety goggles		
No		57 (57.6%)
Sometimes		8 (8.1%)
Yes		34 (34.3%)

SD, standard deviation.
[a]Expressed as mean ± SD.

In the spirometric evaluation to assess lung functions of ceramic workers and their controls forced expression volume in 1 s (FEV1), forced vital capacity (FVC) and % FEV1/FVC ratios were measured. An obstructive pattern was defined as an % FEV1/FVC ratio <75% (27). In the group of workers, 13% displayed % FEV1/FVC ratios less than 75%. Average value of % FEV1/FVC ratios was found to be 81.4 ± 5.3 (mean ± SD) for workers and 81.3 ± 5.2 (mean ± SD) for controls. There were no statistically significant differences between workers and controls regarding to FEV1/FVC ratios.

Exposure measurements

The respirable total dust measurement varied according to the different parts of the plant and range from 0.9 to 4.1 mg/m^3. Glazing was the unit where dust concentrations were highest. The mean concentration of respirable total dust in ceramic workers was 3.6 mg/m^3.

Comet assay

DNA damage in the lymphocytes and the whole blood of the workers were found to be significantly higher than the control group ($P < 0.05$) (Tables 2 and 3). Workers older than 42 years and workers working in the ceramic plant more than 16 years have significantly higher DNA damage in the lymphocytes and the whole blood when compared to the other workers ($P < 0.05$). There were no statistically significant correlation between alcohol usage, smoking and DNA damage. It is interesting that there was no correlation between DNA damage and usage of protective measures of workers in the lymphocytes and the whole blood. On the other hand, according to comprehensive interview, it was learned that these

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Table 2. DNA damage in lymphocytes among workers and control group[a]

Factors	Tail intensity	
	Controls (n = 81)	Workers (n = 99)
	2.6 ± 1.1	4.2 ± 3.8[b]
Age		
18–29	1.3 ± 0.7	4.4 ± 2.4
30–42	1.8 ± 1.3	3.2 ± 2.6
>42	3.0 ± 0.7	11.8 ± 5.0[c]
Smokers	2.3 ± 1.0	3.8 ± 2.9
Non-smokers	2.3 ± 1.1	2.6 ± 1.5
Alcohol consumption		
No	0.0 ± 0.0	3.1 ± 2.1
Yes	2.6 ± 1.0	4.9 ± 4.5
Duration of exposure (years)		
0–5		3.6 ± 1.8
6–10		2.6 ± 2.2
11–15		4.8 ± 4.9
16–20		5.5 ± 4.9[c]
Using protective equipment		
Gloves		
No		4.1 ± 2.8
Sometimes		2.3 ± 1.2
Yes		3.6 ± 2.8
Mask		
No		2.7 ± 2.5
Yes		3.6 ± 2.6
Safety goggles		
No		2.7 ± 2.1
Sometimes		3.8 ± 2.7
Yes		4.0 ± 2.9
Silicosis		5.6 ± 5.0
Suspicion of silicosis		2.8 ± 2.3
Normal		3.2 ± 2.1

SD, standard deviation.
[a]Expressed as mean ± SD.
[b]$P < 0.05$ compared to controls, [c]$P < 0.05$ compared among workers.

Table 3. DNA damage in whole blood among workers and control group[a]

Factors	Tail intensity	
	Controls (n = 81)	Workers (n = 99)
	5.4 ± 2.4	7.4 ± 3.2[b]
Age		
18–29	2.8 ± 1.5	5.8 ± 2.5
30–42	2.7 ± 0.6	7.0 ± 2.5
>42	6.5 ± 1.8	13.5 ± 3.6[c]
Smokers	5.8 ± 2.1	7.2 ± 2.7
Non-smokers	4.9 ± 2.5	6.8 ± 2.4
Alcohol consumption		
No	0.0 ± 0.0	6.8 ± 2.5
Yes	5.4 ± 2.4	7.8 ± 2.7
Duration of exposure (years)		
0–5		6.5 ± 2.9
6–10		7.2 ± 3.3
11–15		8.2 ± 3.4
16–20		10.6 ± 5.8[c]
Using protective equipment		
Gloves		
No		6.5 ± 2.7
Sometimes		7.5 ± 2.2
Yes		6.9 ± 2.6
Mask		
No		7.8 ± 2.1
Yes		6.7 ± 2.6
Safety goggles		
No		7.8 ± 2.3
Sometimes		5.5 ± 3.4
Yes		5.5 ± 2.4
Silicosis		7.9 ± 4.1
Suspicion of silicosis		7.1 ± 2.2
Normal		7.1 ± 2.8

SD, standard deviation.
[a]Expressed as mean ± SD.
[b]$P < 0.05$ compared to controls, [c]$P < 0.05$ compared among workers.

protective equipments were not suitable for better protection. It is also assumed that many workers have not answered the questionnaire correctly regarding the usage of safety measures. Workers with silicosis have slightly higher DNA damage compared to the other workers. This might be explained by the fact that 50% of workers diagnosed as silicosis or suspected have silicosis and 84% of these workers were found to be ILO category 1 which means onset of the disease.

There was a positive significant correlation of DNA damage levels in lymphocytes and whole blood (r = 0.50, $P < 0.05$).

Buccal MN cytome assay (BMCyt assay)

Buccal MN frequency of the workers were found to be significantly higher than the control group ($P < 0.05$) (Table 4). Workers working in the ceramic plant more than 16 years and using alcohol had significantly higher buccal MN frequencies ($P < 0.05$). There were no significant correlation between smoking, age, using protective equipments and buccal MN frequency. Workers with silicosis and suspected to have silicosis had higher buccal MN frequencies compared to the workers, but the differences were not found to be significant.

When evaluating the frequencies of abnormal cells other than MN in buccal mucosa cells, it was shown that CC + KHC and PYC cell frequencies were significantly higher than the control group ($P < 0.05$) (Table 5).

Plasma 8-hydroxydeoxyguanosine (8-oxodG) levels

Plasma 8-oxodG levels of the workers were found to be significantly higher than the control group ($P < 0.05$) (Table 6). Workers working in the ceramic plant more than 16 years and smokers had significantly higher plasma 8-oxodG levels when compared to the other workers ($P < 0.05$). There were correlation between using alcohol, age and 8-oxodG levels, but it was not statistically significant. There was no correlation between using protective equipments and plasma 8-oxodG levels. Workers with silicosis have slightly higher 8-oxodG levels when compared with the other workers.

To determine the most effective parameters on DNA damage in lymphocytes and whole blood, buccal MN frequencies and 8-oxodG levels in plasma of subjects, age, smoking, alcohol consumption and duration of exposure were included to multiple regression analysis (Table 7). Significant effects of duration of exposure were found on all examined parameters. Smoking was found effective on DNA damage in lymphocytes and whole blood, age was found effective DNA damage in lymphocytes and whole blood and 8-oxodG levels in plasma. There was no significant effect of alcohol consumption on results.

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Table 4. Buccal MN frequencies among workers and control group[a]

Factors	MN frequencies (‰)	
	Controls (n = 81)	Workers (n = 99)
Age	3.0 ± 3.4	9.1 ± 8.6[b]
18–29	3.2 ± 3.9	10.8 ± 12.7
30–42	1.1 ± 1.8	8.6 ± 8.6
>42	3.6 ± 3.5	8.0 ± 6.9
Smokers	3.1 ± 1.2	7.6 ± 8.7
Non-smokers	2.9 ± 0.9	9.3 ± 6.4
Alcohol consumption		
No	0.0 ± 0.0	7.4 ± 8.0
Yes	3.0 ± 3.4	11.0 ± 5.0[c]
Duration of exposure (years)		
0–5		4.6 ± 5.2
6–10		8.9 ± 9.4
11–15		5.7 ± 3.9
16–20		11.6 ± 9.3[c]
Using protective equipment		
Gloves		
No		3.4 ± 2.7
Sometimes		8.9 ± 1.2[c]
Yes		8.6 ± 2.8[c]
Mask		
No		4.8 ± 4.9
Yes		8.8 ± 8.0
Safety goggles		
No		11.7 ± 9.5
Sometimes		6.9 ± 6.4
Yes		4.6 ± 3.9
Silicosis		9.7 ± 9.0
Suspicion of silicosis		11.2 ± 11.6
Normal		8.4 ± 7.9

SD, standard deviation.
[a]Expressed as mean ± SD.
[b]$P < 0.05$ compared to controls, [c]$P < 0.05$ compared among workers.

Table 6. Plasma 8-oxodG levels among workers and control group[a]

Factors	8-oxodG (pg/ml)	
	Controls (n = 81)	Workers (n = 99)
Age	33.1 ± 24.8	148.1 ± 2.6[b]
18–29	36.8 ± 27.8	15.3 ± 73.9
30–42	32.2 ± 26.9	141.3 ± 75.4
>42	33.2 ± 24.4	167.4 ± 124.1
Smokers	30.5 ± 24.0	240.2 ± 174.0[c]
Non-smokers	20.8 ± 0.0	163.8 ± 94.6
Alcohol consumption		
No	33.1 ± 24.8	146.8 ± 78.3
Yes	0.0 ± 0.0	167.2 ± 63.4
Duration of exposure (years)		
0–5		120.9 ± 58.4
6–10		161.4 ± 79.3
11–15		173.9 ± 111.1
16–20		225.2 ± 176.3[c]
Using protective equipment		
Gloves		
No		155.0 ± 79.6
Sometimes		128.0 ± 87.5
Yes		131.5 ± 70.4
Mask		
No		141.6 ± 73.5
Yes		151.7 ± 92.3
Safety goggles		
No		143.9 ± 82.7
Sometimes		156.4 ± 76.0
Yes		109.2 ± 63.2
Silicosis		151.6 ± 72.1
Suspicion of silicosis		103.8 ± 106.0
Normal		146.7 ± 81.2

SD, standard deviation.
[a]Expressed as mean ± SD.
[b]$P < 0.05$ compared to controls, [c]$P < 0.05$ compared among workers.

Table 5. Abnormal cell frequencies other than MN in buccal cells[a]

	Controls (n = 81)	Workers (n = 99)
BN	4.8 ± 3.1	4.9 ± 2.8
CC + KHC	5.4 ± 3.3	19.5 ± 13.6[a]
KYL	11.5 ± 11.1	10.9 ± 13.6
PYC	4.3 ± 3.5	10.3 ± 8.9[b]
NBUD	0.4 ± 0.6	0.6 ± 0.8

BN, binucleated; CC, condensed chromatin; KHC, karyorrhectic; KYL, karyolytic; PYC, pyknotic; NBUD, nuclear bud; SD, standard deviation.
[a]Expressed as mean ± SD.
[b]$P < 0.05$ compared to controls.

Discussion

Crystalline silica is one of the most serious occupational hazards. Recent reports estimated that there were 23 million silica-exposed workers in China, over 3 million in India and over 2 million in Europe (28,29). In the ceramic industry, workers are occupationally exposed to free crystalline silica dust during many operations and crystalline silica dust levels vary in different ceramics processing. Silica levels are higher during moulding, in handling and in some mixing jobs and after firing (30). Ceramic workers have elevated risk of chronic silicosis and the prevalence of the disease increased with the cumulative exposure (31). There is a need for reliable biomarkers to predict the likelihood of silicosis and lung cancer development (27). This work was conducted to evaluate the possible genotoxic effects of exposure to inhaled dust, including free silica, in a Turkish ceramic plant. To determine the health status of workers, radiographic chest films and pulmonary function tests were also examined. It is found that DNA damage in blood and lymphocytes, MN, CC + KHC, PYC frequencies in buccal epithelial cells and plasma 8-oxodG levels were increased in silica exposed workers when compared with their controls. Workers working in the ceramic plant more than 16 years have significantly higher DNA damage in lymphocytes and whole blood. Also they have significantly higher buccal MN frequencies and plasma 8-oxodG levels.

Ceramic and pottery workers demonstrated increased cancer risk in Sweden and Italy (32,33). However, there are some epidemiological studies that did not show an excess cancer mortality following exposure to silica (34). A case control study of lung cancer deaths among South Africa gold miners, carefully matched with controls especially for tobacco consumption showed no association between lung cancer and either silicosis or exposure to silica. The authors suggested that the association between silicosis and lung cancer seen in other studies might have been the result of an association between silicosis and smoking (35). However, reports from many countries have shown a high prevalence of lung cancer among workers with silicosis (35–38).

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Table 7. Multiple linear regression analysis of DNA damage in lymphocytes and whole blood, buccal MN frequencies and 8-oxodG levels in plasma of the workers and controls

	DNA damage in lymphocytes	DNA damage in whole blood	Buccal MN frequencies (‰)	8-oxodG levels in plasma (pg/ml)
	B (95 % Cls)			
Age	0.2 (0.0 to 0.1)[a]	0.4 (0.1 to 0.2)[a]	–0.1 (–0.2 to 0.1)	–0.2 (–3.1 to –0.7)*
Alcohol consumption	–0.0 (–1.2 to 0.8)	0.0 (–0.6 to 1.3)	–0.1 (–6.2 to 1.6)	–0.1 (–54.4 to 19.7)
Smoking	–0.2 (–2.7 to 0.2)*	–0.3 (–2.7 to –0.5)*	0.0 (–1.7 to 2.9)	–0.0 (–24.9 to 17.7)
Duration of exposure	0.2 (0.0 to 0.2)*	0.2 (0.0 to 0.2)*	0.3 (0.2 to 0.6)*	0.5 (4.8 to 8.5)*

B, regression coefficient (slope); CIs, confidence intervals.
*Statistically significant ($P < 0.05$).

In relation to silica exposure, only few studies exist in which genotoxicity has been determined. The sister chromatid exchange (SCE) and the chromosomal aberration assay were used in stone crushers, 8-oxodG levels in leukocyte DNA and urine were evaluated in granite workers and in healthy and silicotic coal workers, the comet assay was used in a study among foundry and pottery workers and cytokinesis-block MN assay and SCE assays were performed in coal workers (2–4,7).

The comet assay represents a sensitive measure of DNA damage measured in individual cells. In addition to epidemiological studies, it has been used to measure *in vitro* silica-induced DNA damage in alveolar macrophages (39–41). Similar to our study, Basaran *et al.* (3) investigated the DNA damage by comet assay among foundry and pottery workers and they found significantly higher DNA damage in the lymphocytes of the workers compared to controls. Also, Halder and De (42) showed that silica exposed female mine and quarry workers ($n = 45$) have significant DNA damage in their lymphocytes compared to the their controls by comet assay. Sellappa *et al.* (43) found that construction workers ($n = 96$) showed a significant increase in comet tail length compared to controls with adjustment for smoking habits, tobacco chewing, alcohol consumption and years of exposure. It is also indicated that chronic occupational exposure to cement during construction work was associated with increased levels of DNA damage and repair inhibition.

Cytogenic changes associated with silica exposure have been reported in some studies such as increased number of chromosome aberrations (CA) and SCEs (11). MN may originate from acentric fragments (chromosome fragments lacking a centromere) and/or whole chromosomes (44). Sellappa *et al.* (43) found that construction workers ($n = 96$) have a significant increase in blood MN. Villarini *et al.* (45) showed that tunnel workers have significantly higher MN frequencies in peripheral blood compared to the controls. Although there were no significant differences in the level of primary and oxidative DNA damage was measured by the Fpg- and EndoIII-modified comet assay and frequency of SCE in their study. The MN assay in exfoliated cells is a useful and very promising technique for study of genotoxic effects of different carcinogens and mutagens in human populations. Glass industry workers, sandblasters and stone grinders exposed to crystalline silica had significantly higher nasal MN frequencies (46). It was shown that buccal MN frequencies of mine and quarry workers were significantly higher than the controls (42).

Similar to our study, silica exposed pottery workers in South India had an increase in the total DNA damage evaluated by comet assay. Also, it was showed that they had increased frequency of CA and MN (47).

Peluso *et al.* (48) found that 135 silica-exposed workers employed in pottery, ceramic and marble manufacturing plants as well as in a stone quarry have significantly higher 3-(2-deoxy-β-D-erythropentafuranosyl)pyrimido[1,2-α]purin-10(3H)-onedeoxyguanosine (M1dG) adducts, a biomarker of oxidative stress and peroxidation of lipids, in the nasal epithelium when compared with 118 controls living in Tuscany region, Italy.

In studies that evaluate the relationship between occupational exposure to airborne particulates and pulmonary diseases, early radiographical categories including 0/1 and 1/0 are considered as early stages of pneumoconiosis (49). In this study, nearly 50% of workers were diagnosed with silicosis and 84% displayed silicosis in profusion category 1, 10% silicosis in profusion category 2 and 6% silicosis in profusion category 3, whereas all the controls had normal chest radiographs. These results showed that a majority of the workers displayed onset of silicosis. In Turkey, silicosis ratios were found to be 36, 12 and 7% in the studies of sandblasters, quartz processing workers and ceramic workers, respectively (6,50,51). When evaluating the pulmonary function, there were no statistically significant differences between FEV1/FVC ratios of workers and controls. Although total respirable mean dust levels in the plant were below the accepted limit value (5 mg/m^3) of the occupational exposure in Turkey, the prevalence of silicosis is found to be quite high. It seems that exposure measurement in the plant was not done properly. No data are available about the levels of crystalline silica in dust because only respirable dust measurements are obligatory in Turkey.

In conclusion, the findings of increased DNA damage in peripheral lymphocytes and whole blood, MN frequencies in buccal epithelial cells and plasma 8-oxodG levels of ceramic workers, demonstrate the possibility of genotoxic risk to individuals exposed to chemical mixture in ceramic industry. The workers in this study must be examined in detail to avoid the development of a carcinogenic process. As a recommendation, to reduce silica containing dust exposure and to prevent toxic effects, specific ventilation practices, lowering the limit values as well as using suitable protective equipments should be performed. Our results also show that BMCyt assay seems to be a reliable, rapid and promising method for detecting genetic damage associated with crystalline silica containing dust exposure via inhalation.

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