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Changes in genotoxicity, inflammatory and oxidative stress parameters of workers in marble processing plants

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ABSTRACT

Workers in marble processing plants are at high risk of exposure to high levels of marble dust containing silica, but there are limited studies evaluating the genotoxicity and oxidative stress parameters of workers occupationally exposed to marble dust. In this study, we aimed to clarify how marble dust affects genotoxicity and immunotoxicity mechanisms alongside oxidative stress in the workers in the marble processing plants of Iscehisar, Turkey. The oxidative stress and immune system parameters were determined spectrophotometrically using commercial kits. Genotoxicity was evaluated by Comet and micronucleus (MN) assays in the lymphocytes and buccal cells, respectively. The enzyme activities of superoxide dismutase, catalase, glutathione reductase, glutathione peroxidase, and the levels of glutathione, and Clara cell secretory protein CC16 in workers (n = 48) were significantly lower than in controls (n = 41), whereas the levels of malondialdehyde, 8-oxo-7,8-dihydro-2′-deoxyguanosine, tumor necrosis factor-alpha, interleukin-1beta were significantly higher in workers. DNA damage in workers were significantly higher than in controls and there was a clear correlation between the increase in DNA damage and the duration of exposure. Marble workers had significantly higher MN frequencies when compared to controls. The results indicate the possibility of immunotoxic and genotoxic risks to workers in marble industry.

1. Introduction

The rapid growth of industrialization has caused accidental, occupational, and environmental exposure to various chemical substances that can be allergic, genotoxic, and even carcinogenic. High levels of dust particulates, ranging from 1 to 100 μm, are generated by the process of rock/mineral, marble, timber or fiber material, and dry grain. The occupational exposure of these dust particles can lead to various health and respiratory problems depending on the type and size of dust and duration of exposure (WHO, 1999).

Since ancient times, marble, a natural stone, has been preferred for many purposes such as shelter, protection and art, due to its durability and aesthetic appearance. Turkey constitutes approximately 33% of the world reserve (5.1 billion m^3 of 15 billion m^3 world reserves) and ranks among the noteworthy marble producers in the world. Iscehisar (Afyonkarahisar) is known as one of the most important marble production and processing plants in Turkey (Celik and Sabah, 2008).

Marble is a metamorphic rock composed of calcium carbonate with a crystalline silica of less than 1% (Angotzi et al., 2005). High levels of dust formed during crushing, cutting, sizing, or drilling in marble quarries, can cause negative consequences for environment and human health. Few studies evaluating toxicological exposure to respirable marble dust indicate that it may cause respiratory diseases such as asthma, chronic bronchitis, rhinitis, and damage to lung functions (Angotzi et al., 2005; El-Gammal et al., 2011). It is thought that crystalline silica found in the dust particles has been responsible for these health effects.

Nowadays, it is estimated that there are millions of workers in

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different occupational settings, exposed to respirable crystalline silica (aerodynamic diameter <10 μm). Exposure is in question on about 3–5 million workers in Europe (Matteis et al., 2017); on about 2.3 million workers in the United States (OSHA-a); on more than 10 million workers in the low and middle-income countries like India and China (Leso et al., 2019). As crystalline silica is a cause of many serious diseases and mortalities, it is accepted as a major health problem in developing countries (Leung et al., 2012; Steenland and Ward, 2014). But the surveillance studies are inadequate and the reported rates are thought not to reflect the actual exposure values (Steenland and Ward, 2014).

Many pulmonary diseases such as silicosis, tuberculosis, chronic obstructive pulmonary diseases, lung and other (stomach and throat, etc.) cancers, chronic renal diseases, and autoimmune disorders have been reported to be associated with crystalline silica. The health effects of crystalline silica are mainly due to the accumulation of respirable dust particles in the lungs and the amount of quartz silica in the dust (NIOSH, 2002). Quartz and cristobalite polymorphs of crystalline silica were classified as group 1 "human carcinogen" by International Agency for Research on Cancer (IARC) in 1997 (IARC, 1997). The oxidative stress, immunotoxicity and genotoxicity are thought to play an important role in cancer pathogenesis due to exposure to silica. According to genotoxicity studies carried out with crystalline silica dust exposed-workers, it has been concluded that crystalline silica can cause DNA damage; however, the advanced studies are required in order to interpret the genotoxicity mechanisms (Bonassi et al., 2016; Demircigil et al., 2010; Donmez-Altuntas et al., 2007; Dusinská et al., 2004; Gövercin et al., 2014; Kawami et al., 2000; Marini et al., 2011; Sellappa et al., 2010). Unfortunately, there are not enough studies about the impacts of occupational marble dust exposure on genotoxicity and immunotoxicity.

Oxidative stress is a condition that results from a physiological imbalance between antioxidants and oxidants (free radicals or reactive oxygen/nitrogen species) in favor of oxidants. This imbalance triggers the deterioration of genetic, metabolic, and cellular changes. Antioxidant enzymes including superoxide dismutase (SOD), catalase (CAT), glutathione reductase (GR), and glutathione peroxidase (GPx), and also antioxidants including glutathione (GSH) are useful biomarkers to evaluate the oxidative stress (Davies, 2000). Guanine is the most susceptible nucleic base to oxidative stress caused by reactive oxygen species. 8-oxo-7,8-dihydro-2′-deoxyguanosine (8-oxodG) levels is a convenient marker of oxidative DNA damage and cellular oxidative stress (Pilger and Rüdiger, 2006). Reactive oxygen species may also cause lipid peroxidation by attacking the membrane lipids. Lipid peroxidation leads to formation of aldehydes and ketones including malondialdehyde (MDA), a commonly measured biomarker in human studies. It is documented in experimental and human studies that these products cause damage to the genetic material (Wultsch et al., 2021). Concomitantly, the occupational dusts can induce an inflammatory response that involves alveolar epithelial cells and elements of the immune system, such as, macrophages, neutrophils, lymphocytes, cellular adhesion molecules, cytokines, and chemokines. Proinflammatory cytokines including tumor necrosis factor (TNF)-alpha, and interleukin (IL)-1beta activate functions of inflammatory cells. Hence, these cytokines could be helpful for the evaluation of immunotoxicity (Elsabahy and Wooley, 2013). Additionally, Clara cell secretory protein 16 (CC16), a protein secreted by non-ciliary bronchiolar Clara cells, has been shown to protect the respiratory system against oxidative stress and inflammation. CC16 concentration in serum has recently been proposed as a sensitive marker of acute or chronic disorders of respiratory epithelium (Broeckaert and Bernard, 2000).

The comet (single cell gel electrophoresis) assay is simple, and highly sensitive technique for the determination of genotoxicity (Collins, 2004; Tice et., 2000). Micronucleus (MN) frequency is another biomarker of genetic damage. Buccal micronucleus cytome assay (BMCyt) is a minimally invasive, reliable, rapid, and promising method for the assessment of DNA damage (Bonassi et al., 2011a; Ceppi et al., 2010; Tomas and Fenech, 2011).

The purpose of our study was to evaluate the genotoxicity, oxidative stress, and immune system parameters in workers in marble processing plants (e.g. marble quarries, marble factories, marble workshops) in Iscehisar (Afyonkarahisar), Turkey. The genotoxicity was determined by comet assay in blood samples and MN assay in buccal epithelial cell samples. Besides, SOD, CAT, GR and GPx enzyme activities, and GSH and MDA levels as the indicators of oxidative stress, 8-oxodG levels as an indicator of oxidative stress-related DNA damage, and TNF-alpha, IL-1beta, and CC16 levels as the indicators of inflammatory response were evaluated.

2. Material and methods

2.1. Subjects

The study population consisted of 48 male workers employed in marble processing plants of Iscehisar (Afyonkarahisar), Turkey for at least six months and 41 non-exposed male office workers (controls) of comparable age, sex, lifestyle, smoking habits and living in the same area and with no history of occupational exposure to marble dust or other chemicals (Table 1).

Before the sample collection, a detailed questionnaire (health conditions, medical history, alcohol and smoking habits) was applied. Use of protective mask, years of employment, respiratory symptoms were also recorded (Tables 1 and 2). Subjects who reported active infection, chronic diseases, radiotherapy or chemotherapy were excluded. No alcohol intake was reported for all study group.

All volunteers were informed about the aim of study and their written consent was obtained. This study was approved by the local ethics commission of Hacettepe University. The study was conducted in accordance with the ethical standards in the 1964 Declaration of Helsinki.

2.2. Exposure assessment

For air sampling, particulate-monitoring device Dustmate (Turnkey Instrument Ltd., UK) was used. The measuring range of the device is 0–65000 μg/m^3. The instrument can simultaneously monitor the concentrations of total suspended particles and particles with different diameters (1 μm, 2.5 μm and 10 μm). Samples were collected with the principle of light scattering in accordance with the Turkish Standard (TS) 2361 "Methods for the Measurement of Air Pollution Determination of Concentration of Suspended Matter standard".

2.3. Sample preparation

A total of 18 ml of peripheral blood samples were taken from each volunteer. All blood samples were stored at +4 °C and processed within 6 h 5 ml of blood samples was collected in EDTA containing tubes for the

Table 1
Characteristics of the study population.

	Workers (n = 48)	Controls (n = 41)
Age (years)	37.81 ± 8.50 (21–58)	38.32 ± 7.73 (21–57)
Year of working	9.81 ± 8.33 (0.5–35)	–
Protective mask usage		
Yes	12 (25%)	–
No	36 (75%)	
Smoking status		
Non-smoker	20 (41.67%)	25 (60.98%)
Smoker	28 (58.33%)	16 (39.02%)[a]
Cigarettes/day	16.96 ± 7.12 (10–40)	18.31 ± 3.68 (10–20)

The values are given as the mean ± standard deviation (range). In both groups, individuals who smoked more than ten cigarettes/day for at least 1 year were considered smokers. n: Numbers of individual. [a]p <0.05, compared to controls using z-test.

Table 2
Pulmonary function parameters and pulmonary radiography of the workers exposed to marble dust.

Parameters	Workers		
Pulmonary function tests			
Forced Expiratory Volume in 1 s (FEV1)	59.85 ± 20.63		(15–100)
Forced Vital Capacity (FVC)	53.58 ± 20.36		(13–95)
FEV1/FVC ratio	85.96 ± 18.68		(27–122)
Presence and severity of airway obstruction of workers			
Normal	14	(29.17%)	
Obstructive pattern	1	(2.08%)	
Restrictive pattern	23	(47.92%)	
Mixed obstructive and restrictive	10	(20.83%)	
Chest radiography			
Normal	30	(62.5%)	
Asymmetric (unilateral) lymphadenopathy	11	(22.92%)	
Symmetric (bilateral) lymphadenopathy	3	(6.25%)	
Reticulonodular interstitial pattern	4	(8.33%)	

The values of pulmonary function tests are given as the mean ± standard deviation (range). The values of presence and severity of airway obstruction of workers and chest radiography are given as number of workers (%).

analysis of hemogram parameters. 3 ml of blood samples were allowed to clot for the measurement of serum C-reactive protein (CRP), aspartate aminotransferase (AST), alanine aminotransferase (ALT), total cholesterol (TC), high-density lipoprotein (HDL). The hemograms and serum biochemical parameters were measured immediately. 10 ml of blood samples were collected in sodium heparin containing tube and the plasma was obtained from 5 ml of the heparinized blood sample for the analysis of SOD, CAT, GR, GPx, GSH, MDA, 8-oxodG, TNF-alpha, IL-1beta, and CC16. The plasma samples were stored at −80 °C until the day of analysis. 5 ml of the remaining heparinized blood samples were used to analyze DNA damage. In addition, buccal epithelial cell samples were taken from each volunteer for the determination of MN frequencies.

2.4. Analysis of biochemical parameters

The analysis of total blood counts including white blood cell (WBC), red blood cell (RBC), hemoglobin (HGB), hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), platelet (PLT), red cell distribution width standard deviation (RDW-SD), red cell distribution width (%), platelet distribution width (PDW), mean platelet volume (MPV), platelet-large cell ratio, procalcitonin, nucleated red blood cell (NRBC), neutrophils, monocytes, lymphocytes, eosinophil, basophils, immunoglobulin G levels were performed using the Sysmex XN-2000 hematology autoanalyzer (Sysmex Europe GMBH, Bornbarch 1, 22848, Norderstedt, Germany). The samples were analyzed in duplicate. The results were given as the mean ± standard deviation (range) and expressed as 10^3/μl for WBC, PLT, neutrophils, monocytes, lymphocytes, EO, BASO, IG; 10^6/μl for RBC; g/dl for HGB and MCHC; % for HCT, RDW-CV, P-LCR, PCT and NRBC; femtoliter (fl) for MCV, RDW-SD and MPV; picogram (pg) for MCH.

Inflammation marker (CRP), liver function parameters (AST, ALT), and serum lipid levels (TC, HDL) in the serum samples were determined using the Sysmex XN-2000 hematology autoanalyzer. The samples were analyzed in duplicate. The results were given as the mean ± standard deviation (range) and expressed as mg/dl for CRP, TC, and HDL; U/L for AST and ALT.

2.5. Analysis of oxidative stress and immune parameters

The determination of SOD, CAT, GR, GPx enzyme activities and GSH, MDA, 8-oxodG, TNF-alpha, IL-1beta, and CC16 levels in the plasma samples were carried out spectrophotometrically using assay kits (SOD, CAT, GR, GPx, GSH, MDA from the Cayman Chemical Company (Ann Arbor, MI, USA); 8-oxodG, TNF-alpha, IL-1beta, CC16 from Elabscience (Houston, T, USA)) with manufacturer's directions at 450 nm, 540 nm, 340 nm, 340 nm, 405 nm, 540 nm, 450 nm, 450 nm, 450 nm, and 450 nm, respectively. The samples were analyzed in duplicate. The results were given as the mean ± standard deviation (range) and expressed as U/ml for SOD; nmol/min/ml for CAT, GR, and GPx; μM for GSH and MDA; ng/ml for 8-oxodG; pg/ml for TNF-alpha, IL-1beta, and CC16.

2.6. Alkaline single-cell gel electrophoresis (COMET) assay

The basic alkaline single-cell gel electrophoresis technique (Comet assay) was performed as described previously (Aydin et al., 2013, 2019; Collins, 2004). After the isolation of peripheral blood mononuclear cells (PBMCs) from heparinized blood samples, the cell concentrations were adjusted to approximately $2x10^5$ cells/ml in PBS. Cell viability checked by trypan blue was higher than 85% in all cases. The cells were embedded in agarose on a microscope slide and lysed with fresh cold lysing solution (2.5 M NaCl,100 mM EDTA, 100 mM Tris,1% sodium sarcosinate, pH 10), with 1% Triton X-100 and 10% DMSO for 1 h at 4 °C to form nucleoids containing supercoiled loops of DNA linked to the nuclear matrix. Then, they were removed from the lysing solution, drained, and left in the electrophoresis solution (1 mM sodium EDTA and 300 mM NaOH, pH 13) for 20 min at 4 °C to allow the unwinding of DNA and expression of alkali-labile damage. Electrophoresis was carried out for 20 min at 4 °C with a current of 25 V (300 mA). After electrophoresis, the slides were neutralized and then incubated in 50%, 75% and 98% alcohol for 5 min, successively. The dried microscopic slides were stained with ethidium bromide and covered with a cover-glass prior to analysis with a Leica® 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 4.0, Kinetic Imaging Ltd., Liverpool, UK) to determine the extent of DNA damage after electrophoretic migration of DNA fragments in the agarose gel. One-hundred nucleoids (comets) from each of duplicate slides were examined at 400x and the results were expressed as the percentage of DNA in tail (tail intensity).

2.7. Buccal micronucleus cytome (BMCyt) assay

The BMCyt assay for the determination of chromosomal genetic damage was performed according to the procedure described by Thomas and Fenech (2011) and Bonassi et al. (2011b). Volunteers were asked to rinse their mouths with distilled water and exfoliated buccal epithelial cells were collected by scraping from the mucosa of both cheek with a toothbrush. The cells were shaken in a centrifuge tube containing 10 ml of PBS. The cell suspensions were centrifuged at 1200 rpm for 5 min to remove bacteria and cell debris. The supernatant was discarded, and the procedure was repeated twice. For each sample, the cell suspension was spread on two clean and coded slides, air-dried, and then fixed in 80% methanol for 15 min. The cells were stained with acridine orange dye. For staining, the slides were covered with 0.1% acridine orange (CAS no: 10,127,023; Sigma-Aldrich, St. Louis, Missouri, USA) and incubated for 15 min in the dark (Cao et al., 2002; Thomas and Fenech, 2011).

A total of 2000 cells per individual were classified and scored using fluorescence microscopy (Leica Microsystems DM2500, Wetzlar, Germany) equipment with a 440–490 excitation and 520 nm emission filters at 400x. Micronuclei were evaluated according to the scoring criteria described by Tolbert et al. (1992). Basal cells, binuclear cell, nuclear buds, pyknotic cell, condensed chromatin, karyorrhectic cell and karyolitic cells were excluded from evaluation of micronucleus-included cells (Fig. 1) (Thomas and Fenech, 2011).

2.8. Statistical analysis

Analysis of data was performed using the program SPSS 20.0 for Windows. The normality of distribution was checked by the

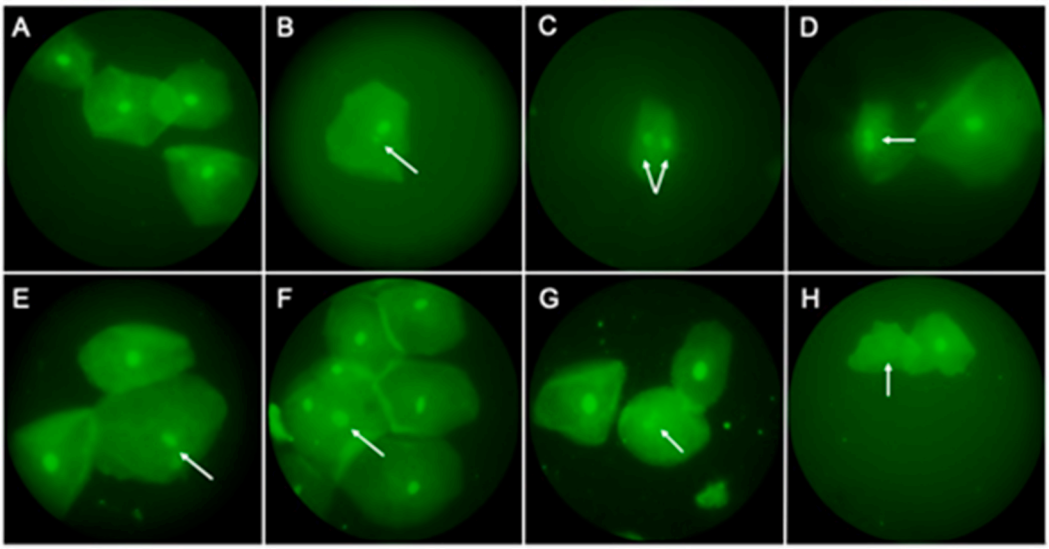

Fig. 1. Various cells in exfoliated buccal cells. A) Basal cells, B) Micronucleus (MN)-included cell, C) Binuclear cell, D) Nuclear Buds (broken egg), E) Pyknotic cell, F) Condensed chromatin, G) Karyorrhectic cell, H) Karyolitic cell (oil-immersion 40x objective, acridine orange stain). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

Kolmogorov-Smirnov test. The homogeneity of the variance was verified by the Levene test. The results were expressed as the mean ± standard deviation (SD) for continuous variables and the number of cases percent (%) for categorical variables. The differences among the groups with normal distribution were determined by the one-way variance analysis (ANOVA) test. 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 and Kruskal-Wallis test for more than two groups. The magnitude of linear relationship was calculated by Pearson correlation analysis. For statistical analysis of BMCty assay results, the z-test was applied for the frequency of MN. The *p*-value of less than 0.05 was considered as statistically significant.

3. Results

3.1. Characteristics of the study population

The characteristics (age, duration of exposure, protective mask usage (dust particle mask) and smoking habits) of the study groups were shown in Table 1. The mean ages of workers and controls were 37.81 ± 8.50 years (range 21–58) and 38.32 ± 7.73 years (range 21–57), respectively. The mean working duration of workers was 9.81 ± 8.33 years (range 0.5–35). Only 12 (25%) workers used protective mask. The number of smokers in the workers (58.33%) (n:28) was significantly higher than smokers in the control group (39.02%) (n:16) ($p < 0.05$). The average consumption of cigarettes in the workers and in the controls were 16.96 ± 7.12 cigarettes/day and 18.31 ± 3.68 cigarettes/day, respectively. No abnormalities in the ear and nose functions of the workers and the controls were noted. Results of pulmonary function in the workers exposed to marble dust were given in Table 2. The normal value of Forced Expiratory Volume (FEV1)/Force Vital Capacity (FVC) ratio in a healthy individual was 75–80%. An obstructive pattern was defined of FEV1/FVC ratios <75% (GINA, 2019). In the workers, the mean FEV1/FVC ratio was found to be normal (85.96 ± 18.68%). However, the percent values of FEV1 and FVC (59.85 ± 20.63% and 53.58 ± 20.36%, respectively) individually were lower than the normal values (>80%). Thirty-four workers (71.83%) had obstructive, restrictive, or mixed pattern according to the presence and severity of airway obstruction as evaluated by FEV1 and FVC whereas 14 workers (29.17%) were found to have normal values. The chest radiography of 30 workers (62.5%) and all pulmonary functions of the control group were normal.

3.2. Assessment of exposure in workers

The concentrations of airborne particulate matter (Particulate matter 1 (PM1), PM2.5, PM10 and total suspended particulate (TSP)) were measured in air samples taken from seven different units including maintenance, polishing, sizing, tumbled, cutting, and carpentry units (Table 3). Results were compared with the permissible exposure limit (PEL) value determined by Occupational Safety and Health Administration (OSHA). In general, PM10 levels (2117.5 ± 1644.5 μg/m^3) were higher than PM2.5 (178.8 ± 140.1 μg/m^3) and PM1 (91.4 ± 125.1 μg/m^3). The mean TSP levels was found to be 2574 ± 1537 μg/m^3 (540.8–4870.4). In the sizing and cutting units, 23 workers (total 47.9%) were exposed to 4553.5 ± 258.4 μg/m^3 (4237–4870) dust, which are close to the limit values of 5000 μg/m^3.

3.3. Biochemical parameters

The biochemical parameters of the study population were given in Table 4. There were no significant differences in all biochemical parameters except serum total cholesterol levels between workers and controls ($p > 0.05$). Total cholesterol levels in the control group were found to be significantly higher than the worker group ($p < 0.05$).

3.4. Oxidative stress and immune parameters

The enzyme activities of SOD, CAT, GR, GPx and the levels of GSH, MDA, 8-oxodG, TNF-alpha, IL-1beta, and CC16 in plasma samples were given in Table 5. The enzyme activities of SOD, CAT, GR, GPx and the levels of GSH and CC16 in the workers were significantly lower than in the control group ($p < 0.05$). MDA, 8-oxodG, TNF-alpha, and IL-1beta levels in the worker group were significantly higher than in the control group ($p < 0.05$). Workers working in the marble processing plant more than 10 years had significantly lower GSH and CC16 levels and higher MDA, TNF-alpha, and IL-1beta levels ($p < 0.05$). It was found that as the duration of exposure increased, GSH (regression coefficient (r) = -0.516) and CC16 (r = −0.635) levels decreased, however MDA (r = 0.406), TNF-alpha (r = 0.654), and IL-1beta (r = 0.621) levels increased. There were no correlations between the duration of exposure and SOD,

Table 3
Dust exposure in the marble processing plants.

	n	Mask usage	TSP (μg/m^3)	PM10 (μg/m^3)	PM2,5 (μg/m^3)	PM1 (μg/m^3)	Limit value (μg/m^3)
Maintenance	3	1 (33.3%)	540.8	211.6	46.4	20.1	5000.0
Polishing	12	5 (41.7%)	2760.3	2124.8	171.9	48.1	5000.0
Sizing	5	0 (0.0%)	4237.7	3717.2	186.1	69.5	5000.0
Tumbled	6	3 (50.0%)	2389.1	2528.2	235.8	83.3	5000.0
Cutting	18	3 (16.7%)	4870.4	4640.0	450.0	368.5	5000.0
Carpentry	1	0 (0.0%)	1820.6	926.7	35.2	5.0	5000.0
Selection	3	0 (0.0%)	1401.3	673.8	126.3	45.1	5000.0
Mean			**2574.3**	**2117.5**	**178.8**	**91.4**	
±SD			**1537.7**	**1644.5**	**140.1**	**125.1**	

The values of mask usage are given as number of workers (%). n: Numbers of workers; TSP: Total suspended particles; PM: Particulate matter. SD: Standard deviation.

CAT, GR, GPx, and 8-oxodG levels.

Mask usage did not change the oxidative stress and immune parameters in the workers. Smoking also did not affect these parameters in the workers and the controls.

3.5. DNA damage

DNA damage in the lymphocytes expressed as DNA tail intensity (% DNA in the tail) were shown in Table 6. DNA damage in the workers were found to be significantly higher than in the control group ($p < 0.05$).

DNA damage in young (19–40 years) and older (41–60 years) workers and also smoker and non-smoker worker groups were higher when compared to their control groups ($p < 0.05$). However, in both workers and controls, there were no significant differences between the older and young workers and also the smokers and the non-smokers.

Tail intensity were 1.46 ± 0.54 (range 0.58–2.45), 1.43 ± 0.63 (range 0.23–2.73), and 2.15 ± 0.81 (range 0.70–3.38) in workers with short, medium and long duration of exposure, respectively. As the duration of exposure increased, DNA damage was found to be significantly higher in workers ($p < 0.05$), but there were no significant differences between short and medium duration ($p > 0.05$). Protective mask usage did not decrease DNA damage in workers. But the percentage of workers using protective masks is only 25% of the study group.

3.6. Buccal micronucleus assay

MN frequencies in the buccal exfoliated cells were shown in Table 7. Buccal MN frequency of the workers (8.9 ± 4.7, range 1–18) were found to be significantly higher than the control group (5.1 ± 2.8, range 1–11) ($p < 0.05$).

The MN frequency of young workers was higher when compared to its controls ($p < 0.05$), however there were no significant differences in the workers and controls for middle age group. In the workers, MN frequency was not different between the young and middle age group ($p > 0.05$), however it increased in the middle age group when compared to the young age group ($p < 0.05$).

Smoking seemed to increase MN frequencies significantly both in the workers and controls since MN frequencies were higher in the smoking workers compared to the non-smoking workers ($p < 0.05$). For controls, MN frequencies were also higher in the smokers compared to the non-smokers ($p < 0.05$).

MN frequencies were 6.33 ± 4.04 (range 2–12), 9.34 ± 4.31 (range 3–17), and 11.56 ± 4.85 (range 1–18) in the workers with short, medium, and long duration of exposure, respectively. There was correlation between the duration of exposure and MN frequencies. It was found that as the exposure time increased, MN frequencies increased. MN frequencies in short-time workers (6 months-4.5 years) were found to be significantly higher than in medium-term workers (5–15 years) and long-time workers (16–35 years) ($p < 0.05$). In addition, there were significant differences between short (6 months-4.5 years) and medium duration (5–15 years) in MN frequencies ($p < 0.05$).

MN frequencies were higher in the workers using protective masks when compared to non-users ($p < 0.05$). Contrary to expectations, using protective mask did not affect MN frequencies and the confusing result may be due to the factors including age and smoking that increase the frequency of MN. Also, the number of workers using protective masks were lower than the workers who were not using masks.

4. Discussion

In the present study, we focused on the possible genotoxicity, immunotoxicity and oxidative stress parameters in workers in marble processing plants in Iscehisar (Afyonkarahisar), Turkey.

Turkey constitutes approximately 33% of the world reserve (5.1 billion m^3 of 15 billion m^3 world reserves) and ranks among the noteworthy marble producers in the world. Iscehisar (Afyonkarahisar), one of Turkey's most important marble production and processing region, is known worldwide in different types and quality of the marble (Celik and Sabah, 2008).

High levels of respirable dusts (<10 μm) are produced while cutting, sizing, polishing, and smoothing marble. Occupational dusts exposure from marble industry is known to cause important health problems in workers. The toxic effects of respirable dusts vary according to the number of dust particles, their diameter, chemical composition and aerodynamic properties. Marble dust consists mainly of calcium carbonate and contains crystalline silica in different proportions less than 1%. Crystalline silica may cause various respiratory diseases that can range from irritation to lung cancer (Angotzi et al., 2005). Although the silica content is not very high in marble dust, it is assumed that silica may be responsible for the health effects of marble dust.

The pulmonary system toxicity related to marble dust exposure has been demonstrated in some clinical studies (Orman et al., 2002; Sezgi et al., 2012; Soysal et al., 2006; Yıldırım et al., 2016); however, the studies on the effects of occupational marble dust exposure on genotoxicity and immunotoxicity are very limited. In the present study, a detailed research was conducted in order to understand how marble dust affects genotoxicity and immunotoxicity mechanisms along with oxidative stress parameters.

With the aim of preventing the risks in the workplace environment, the limit values for respirable dusts were determined as 5000 μg/m^3 by OSHA and as 3000 μg/m^3 by American Conference of Governmental Industrial Hygienists (ACGIH) (OSHA-b). OSHA determined the permissible exposure limit (PEL) value as 50 μg/m^3 and the National Institute for Occupational Safety and Health (NIOSH), the recommended exposure limit (REL) value as 50 μg/m^3 for crystalline silica. ACGIH determined the time weighted average (TWA) value as 25 μg/m^3 for crystalline silica (OSHA-c). In our study, the concentrations of airborne particulate matter (PM1, PM2.5, PM10 and TSP) were measured at seven different units including maintenance, polishing, sizing, tumbled, cutting, carpentry, and selection units in the marble processing plants. Turkey has a very diverse and large amounts of marble reserves, but it is not a common procedure to determine the silica content in the ambient

Table 4
Biochemical parameters.

Parameters	Workers (n = 48)		Controls (n = 41)		p
WBC (10^3/μl)	7.91 ± 1.62	(4.87–11.07)	7.82 ± 1.69	(4.37–12.72)	0.809
RBC (10^6/μl)	5.35 ± 0.37	(4.66–6.67)	5.35 ± 0.55	(4.27–6.99)	0.942
HGB (g/dl)	15.48 ± 1.05	(12.5–18)	15.35 ± 1.10	(12.1–17.7)	0.578
HCT (%)	45.06 ± 2.82	(33.8–49.2)	45.12 ± 3.13	(36.2–51.3)	0.925
MCV (fl)	85.0 ± 5.39	(64.5–93.2)	84.71 ± 5.93	(62.5–94.2)	0.813
MCH (pg)	29.07 ± 2.44	(20.2–32)	28.85 ± 2.35	(19.9–32.3)	0.672
MCHC (g/dl)	34.15 ± 1.29	(31.4–36.8)	34.03 ± 0.94	(31.8–36.5)	0.625
PLT (10^3/μl)	225.2 ± 60.29	(108–388)	242.7 ± 43.37	(142–334)	0.122
RDW-SD (fl)	38.98 ± 2.13	(34.3–43.6)	39.56 ± 2.46	(34.5–44.2)	0.242
RDW-CV (%)	12.80 ± 1.11	(11.7–17.5)	13.08 ± 1.30	(11.5–18.7)	0.286
PDW (fl)	13.47 ± 1.98	(10.1–19.6)	13.29 ± 2.01	(10.4–20.4)	0.675
MPV (fl)	10.92 ± 0.84	(9.3–12.7)	10.53 ± 1.69	(1.4–13)	0.193
P-LCR (%)	32.07 ± 8.07	(0.9–48.04)	31.62 ± 6.87	(20.7–48.3)	0.780
PCT (%)	0.24 ± 0.06	(0.13–0.44)	0.26 ± 0.05	(0.16–0.35)	0.203
NRBC (%)	0 ± 0	(0–0.01)	0 ± 0	(0-0)	0.160
Neutrophils (10^3/μl)	4.83 ± 1.31	(2.49–8.54)	4.38 ± 1.36	(2.09–7.85)	0.119
Monocytes (10^3/μl)	0.52 ± 0.17	(0.33–1.32)	0.60 ± 0.13	(0.38–0.79)	0.095
Lymphocytes (10^3/μl)	2.33 ± 0.69	(1.48–4.08)	2.57 ± 0.74	(1.27–4.52)	0.115
EO (10^3/μl)	0.17 ± 0.12	(0.03–0.57)	0.21 ± 0.10	(0.03–0.48)	0.139
BASO (10^3/μl)	0.05 ± 0.02	(0.01–0.13)	0.05 ± 0.02	(0.02–0.11)	0.826
IG (10^3/μl)	0.03 ± 0.02	(0.01–0.11)	0.03 ± 0.01	(0.01–0.07)	0.677
CRP (mg/dl)	0.14 ± 0.16	(0–0.4)	0.15 ± 0.17	(0–0.6)	0.746
AST (U/l)	21.56 ± 4.14	(13.9–33.5)	20.21 ± 5.91	(11.6–41.4)	0.225
ALT (U/l)	21.68 ± 9.71	(10.1–56.3)	25.90 ± 17.09	(9.1–89.4)	0.167
TC (mg/dl)	158.7 ± 41.19	(90.8–273.6)	176.5 ± 32.90	(113.9–273.6)[a]	0.026
HDL (mg/dl)	39.67 ± 7.14	(27.5–57.5)	41.77 ± 11.38	(25.1–83.2)	0.310

The values are given as the mean ± standard deviation (range). [a]p <0.05, compared to controls. WBC: White blood cell; RBC: Red blood cell; HGB: Hemoglobin; HCT: Hematocrit; MCV: Mean corpuscular volume; MCH: Mean corpuscular hemoglobin; MCHC: Mean corpuscular hemoglobin concentration; PLT: Platelet; RDW-SD: Red cell distribution width standard deviation; femtoliter (fl); RDW-CV: Red cell distribution width; PDW: Platelet distribution width; MPV: Mean platelet volume; P-LCR: Platelet-large cell ratio; PCT: Procalcitonin; NRBC: Nucleated RBC; EO: Eosinophil; BASO: Basophils; IG: Immunoglobulin G; CRP: C-reactive protein; AST: Aspartate aminotransferase; ALT: Alanine aminotransferase; TC: total cholesterol; HDL: High-density lipoprotein.

air of marble dust-working areas. However, according to the study involving marble quarrying in Tuscany located in the Apuanian Alps, crystalline silica in marble dust is less than 1% (Angotzi et al., 2005). Located in the Alpine zone (same as Tuscany) where the world's richest natural stone formations, the silica content of marble dust is assumed to be less than 1%. In our workplace, PM10 levels (2117.5 ± 1644.5 μg/m^3) were higher than PM2.5 (178.8 ± 140.1 μg/m^3) and PM1 (91.4 ± 125.1 μg/m^3). The mean of TSP levels was 2574 ± 1537 μg/m^3 (540.8–4870.4) that was lower than the limit values of OSHA (5000 μg/m^3). However, 23 workers (47.9%) working in sizing and cutting areas, were exposed to high TPS levels (4553.5 ± 258.4 μg/m^3

Table 5
Oxidative stress and immune parameters.

Parameters	Workers (n = 48)		Controls (n = 41)		p
SOD (U/ml)	0.26 ± 0.09	(0.12–0.47)	0.39 ± 0.10	(0.22–0.67) [a]	<0.001
CAT (nmol/min/ml)	71.25 ± 18.35	(32.94–113.85)	99.17 ± 13.98	(74.91–125.38) [a]	<0.001
GR (nmol/min/ml)	26.17 ± 5.86	(15.03–38.82)	34.63 ± 12.08	(17.42–79.46) [a]	<0.001
GPx (nmol/min/ml)	125.0 ± 14.99	(90.42–161.89)	137.0 ± 32.23	(97.71–298.40) [a]	0.033
GSH (μM)	8.68 ± 3.47	(2.98–16.04)	12.43 ± 4.38	(5.21–23.59) [a]	<0.001
MDA (μM)	2.60 ± 0.60	(1.48–3.96)	2.07 ± 0.58	(1.21–3.45) [a]	<0.001
8-OHdG (ng/ml)	32.92 ± 8.07	(19.29–52.36)	24.12 ± 7.03	(8.18–42.16) [a]	<0.001
TNF-alpha (pg/ml)	14.73 ± 7.62	(7.81–38.69)	10.66 ± 2.63	(7.87–17.86) [a]	<0.001
IL-1beta (pg/ml)	6.14 ± 5.78	(1.70–25.37)	3.43 ± 1.93	(1.89–10.24) [a]	0.003
CC16 (pg/ml)	528.6 ± 150.0	(259.2–980)	901.0 ± 247.0	(598.8–1401.2) [a]	<0.001

The values are given as the mean ± standard deviation (range). [a]p <0.05, compared to controls. SOD: superoxide dismutase; CAT: catalase; GR: glutathione reductase; GPx: glutathione peroxidase; GSH: glutathione; MDA: malondialdehyde. TNF-alpha: tumor necrosis factor-alpha; IL-1beta: interleukin-1beta; CC16: clara cell secretory protein 16.

Table 6
DNA damage in the lymphocytes of workers exposed to marble dust and controls.

	Tail intensity (% DNA in the tail)			
Factors	Workers (n = 48)		Controls (n = 41)	
	1.59 ± 0.69	(0.23–3.38)	0.95 ± 0.29	(0.43–1.52) [a]
Age				
Younger workers (19–40)	1.57 ± 0.68	(0.23–3.38)	0.92 ± 0.28	(0.43–1.31) [a]
Older workers (41–60)	1.64 ± 0.74	(0.70–2.72)	1.01 ± 0.32	(0.56–1.52) [a]
Smoking				
Smokers	1.60 ± 0.74	(0.23–3.38)	0.91 ± 0.31	(0.43–1.52) [a]
Non-smokers	1.59 ± 0.65	(0.70–2.72)	0.98 ± 0.29	(0.56–1.49) [a]
Duration of exposure (years)			–	
Short duration (0.5–4.5)	1.46 ± 0.54	(0.58–2.45)		
Medium duration (5–15)	1.43 ± 0.63	(0.23–2.73)		
Long duration (16–35)	2.15 ± 0.81	(0.70–3.38) [b]		
Using protective mask			–	
Yes	1.66 ± 0.69	(0.70–2.52)		
No	1.57 ± 0.70	(0.23–3.38)		

The values are given as the mean ± standard deviation (range). [a]p <0.05, compared to controls; [b]p < 0.05, long exposure duration compared to short and medium exposure duration.

Table 7
MN frequencies in buccal exfoliated cells of workers exposed to marble dust and controls.

	MN frequencies (2000)			
Factors	Workers (n = 48)		Controls (n = 41)	
	8.90 ± 4.70	(1–18)	5.10 ± 2.80	(1–11) [a]
Age				
Younger workers (19–40)	8.88 ± 4.70	(2–17)	4.06 ± 2.18	(1–11) [a, b]
Older workers (41–60)	8.84 ± 4.74	(2–18)	6.83 ± 2.88	(2–11)
Smoking				
Smokers	10.34 ± 4.7	(2–18) [c]	6.85 ± 2.68	(3–11) [a, c]
Non-smokers	6.80 ± 3.83	(1–13)	3.94 ± 2.21	(1–10) [a]
Duration of exposure (years)			–	
Short duration (0.5–4.5)	6.33 ± 4.04	(2–12)		
Medium duration (5–15)	9.34 ± 4.31	(3–17) [d]		
Long duration (16–35)	11.56 ± 4.85	(1–18) [e]		
Using protective mask			–	
Yes	11.15 ± 4.40	(4–18)		
No	8.10 ± 4.55	(2–17)		

The values are given as the mean ± standard deviation (range). [a]$p < 0.05$, workers compared to controls. [b]$p < 0.05$, younger age group compared to older age group. [c]$p < 0.05$, smokers compared to non-smokers. [d]$p < 0.05$, medium exposure duration compared to short exposure duration. [e]$p < 0.05$, long exposure duration compared to short and medium exposure duration.

(4237–4870)) that were higher than ACGIH limits (3000 μg/m^3) and also very close to the limit values of OSHA.

OSHA confirmed that between the years of 1980–1992, respirable crystalline silica levels in 48% of 255 workplaces were above the PEL (Freeman and Grossman, 1995). It was stated that respirable crystalline silica levels exceeded the specified limits, especially in the work areas such as rock drilling and sandblasting (Castranova and Vallyathan, 2000). Because of the lack of a definite treatment of silica-induced diseases; prevention, implementing the necessary regulations and inspection of the workplaces for potential risks should be targeted. In addition, occupational exposure to silica was predicted to cause 440,000 cancer cases between 2010 and 2069 in the EU if the permitted exposure limits will not be reduced (Cherrie et al., 2011).

In the present study, no abnormalities were found in ear and nose functions. Pulmonary functions of control group and also the mean FEV1/FVC in workers was found to be normal (85.96 ± 18.68%), consistent with the previous studies (Anlar et al., 2018). However, FEV1 and FVC values (59.85 ± 20.63% and 53.58 ± 20.36%, respectively) individually were lower than the normal values (>80%). 14 workers (29.17%) were healthy, 34 workers (71.83%) had obstructive, restrictive or mixed pattern according to presence and severity of airway obstruction evaluated by FEV1 and FVC. The chest radiography of 30 workers (62.5%) were normal, however 18 workers (37.5%) had abnormal chest radiography including symmetric or asymmetric lymphadenopathy and reticulonodular interstitial pattern. Pulmonary functions were abnormal in 16 of 22 workers who worked for more than 10 years. These results showed that a majority of workers displayed onset of obstructive and/or restrictive pulmonary abnormalities. It appears that respiratory functions were impaired with an exposure time of more than 10 years. Silicosis was not diagnosed in our workers. In a study of conducted on 67 workers, silicosis ratio was found to be 12% of workers working in quartz processing plants in Turkey and it was recommended to take precautions to reduce dust in occupational areas (Polatlı et al., 2001).

In a study conducted in workers (n = 110) in a marble processing plant in Diyarbakır, Turkey with a mean age of 33.4 ± 6.3 years old, the respiratory symptoms, respiratory functions, and chest radiography findings related to marble dust exposure were evaluated. The workers were grouped according to dust concentrations in working air (office 1.7 mg/m^3; tile cutting 4.6 mg/m^3; block cutting 5.2 mg/m^3; polishing mg/m^3). It was found that symptoms such as cough and sputum were observed more frequently in workers exposed to high concentration of dust compared to the group which exposed to low dust concentration (office) ($p < 0.05$). In addition, FEV1, FEV1/FVC, forced expiratory flow (FEF) 25–75% were found to be significantly lower than control group. Although higher pathological findings were found on chest radiography in the groups with high dust concentrations, the difference was not statistically significant. It has been reported that chest radiography pathologies occur more frequently in workers working for more than ten years in the marble processing plants ($p < 0.001$) (Sezgi et al., 2012).

Similar to our study, the negative effects of occupational marble dust on respiratory functions were shown in the studies conducted on 236 male workers in Afyonkarahisar, Turkey (Orman et al., 2002) and 50 male workers in Aydın, Turkey (Soysal et al., 2006). In conclusion, the intensity and duration of marble dust exposure in workers are associated with respiratory symptoms and chest radiography findings.

Oxidative stress is a phenomenon caused by an imbalance between production and accumulation of oxygen reactive species (ROS) in cells and tissues, which is the ability of a biological system to detoxify these reactive products. High dose and/or inadequate removal of ROS, especially superoxide anion, results in oxidative stress. Superoxide anion is dismutated by SODs to H2O2 that is catalyzed to H2O by catalase, peroxiredoxins, or GPx. GPx catalyzes the reduction of various hydroperoxides (e.g., H2O2) to H2O via oxidation of reduced GSH into its disulfide form (GSSH). GRx catalyzes the reduction of glutathione disulfide (GSSG) to the sulfhydryl form glutathione (GSH), which is a critical molecule in resisting oxidative stress and maintaining the reducing environment of the cell. ROS also represent a component of the innate immune system, and they are not only involved in the respiratory burst of neutrophils, but also signal inflammatory cell chemotaxis into sites of inflammation (Gupta et al., 2014). TNF-alpha is also a critical cytokine, which plays a pivotal role in the pathogenesis of immune disorders and tumor development. The roles of inflammatory cytokines and the immune response in cancer remain paradoxical. In the case of TNF, there is undisputed evidence indicating both procarcinogenic and anticarcinogenic activities (Carbone and Yang, 2011). IL-1beta is a potent pro-inflammatory cytokine that is crucial for host-defense responses to infection and injury (Lopez-Castejon and Brough, 2011).

In our study, the enzyme activities of SOD, CAT, GR, GPx and the levels of GSH, and CC16 in the workers were significantly lower than in control group ($p < 0.05$). MDA, 8-oxodG, TNF-alpha, and IL-1beta levels in the workers were significantly higher than in controls ($p < 0.05$). Strong correlations were found between the duration of exposure and the levels of GSH, CC16, MDA, TNF-alpha, and IL-1beta. As the duration of exposure increased (more than 10 years), GSH and CC16 levels decreased; however, MDA, TNF-alpha, and IL-1beta levels increased. The decrease in CC16 level indicates that workers may be incapable of protecting the respiratory system against oxidative stress and inflammation. There were no correlations between the duration of exposure and the levels of SOD, CAT, GR, GPx, and 8-oxodG. Mask usage and smoking did not change the oxidative stress and immune parameters. No significant differences were found in all biochemical parameters except serum total cholesterol levels. Similar to our findings, Anlar et al. (2018) reported that plasma 8-oxodG levels in ceramic workers (n = 99) exposed to complex mixture of chemicals mainly crystalline silica were higher compared to its controls. A significant reduction of serum CC16 has also been found in miner workers (n = 86) exposed to crystalline silica. They concluded that the decreases in the serum CC16 level probably reflect early toxic effects of crystalline silica particles on the respiratory epithelium. (Bernard et al., 1994; Gu et al., 2021). Crystalline silica can lead to oxidative stress with the increases in the expression of antioxidant enzymes such as GPx, SOD and the enzyme inducible

nitric oxide synthase. In addition, generation of oxidants induces cell signaling pathways such as extracellular signal-regulated kinase phosphorylation, MAPK/ERK kinase and increases the production of inflammatory cytokines (TNF-alpha, IL-1beta, etc.), and the activation of specific transcription factors (NF-kB, AP-1 etc.) (Fubini and Hubbard, 2003).

In a study (n = 48, male), the levels of neopterin, a biomarker of the activation of the cell mediated immune system and oxidative stress, were examined in the workers exposed to marble dusts. Neopterin levels in urine were reported to be significantly higher in workers ($p < 0.001$). and crystalline silica in marble dust was found to be responsible for the increased levels of neopterin (Gaballah et al., 2015).

Autoimmune diseases have been reported in workers in marble processing plants: Erasmus syndrome (sclerosis accompanied by crystal silica exposure) has been reported in a marble worker of 70 years old in Italy (Bello et al., 2015). In addition, Caplan syndrome (rheumatoid arthritis accompanied by crystal silica exposure) has been reported in a 45-year-old worker with history of 7 years marble dust exposure (Rozin and Toledano, 2013). The most acceptable theory that explains the relationship between autoimmune disorders of the chronic exposure of crystalline silica, is the corruption of apoptotic mechanism in lymphocytes. In this case, Fas protein, expressed in the cell membrane of lymphocytes and belongs to the TNF receptor family, is considered to be the trigger of the caspase cascade leading to DNA fragmentation (Nagata, 1994; Peng, 2006).

Only few studies have reported genotoxicity in relation to occupational exposure to marble dust and/or silica containing dusts (Borm et al., 2018; Wultsch et al., 2021). The genotoxicity in marble dust-exposed workers may be caused directly by silica particles and/or by free radicals released from activated alveolar macrophages and neutrophils following silica or particulate marble dust inhalation. In our study, DNA damage in the PMNCs of marble workers were found to be significantly higher than in the control group and there was a clear correlation between the increased DNA damage and the duration of exposure. Workers working in marble processing plants more than 16 years had significantly higher DNA damage when compared to the workers working shorter duration. Similar to our study, Başaran et al. (2003) found that DNA damage in the lymphocytes of foundry and pottery workers who were exposed silica was significantly higher when compared to control group using comet assay. Interestingly, in our study, protective mask usage did not affect DNA damage in workers. But the number of workers using masks in the study was quite low, it is thought that the result was not meaningful. The percentage of workers using protective masks is only 25% of the total workers. There is also a doubt in the answers of workers that claimed that they have been using protective masks, it is assumed that the questionnaire was not answered correctly.

Oxidative DNA damage was found to be increased in the nasal epithelium of workers (n = 135) in pottery, ceramics, and marble processing plants in Tuscany-Italy. It has been suggested that occupational silica exposure may cause genotoxic damage that can cause important health problems in workers (Peluso et al., 2015). In systematic review and meta-analysis which focused on MN formation in buccal cells, the risk estimates strongly suggested that occupational genotoxic exposures, especially to silica and formaldehyde increase the MN frequencies. It is also suggested that the frequency of buccal MN could be useful as biomarkers of cancer risk in exposed workers (Hopf et al., 2019).

In our study, we determined MN frequencies in the buccal exfoliated cells of workers exposed to marble dusts containing crystalline silica. Our results showed that marble workers had significantly higher frequencies of MN when compared to controls. Increased MN frequencies were related to the increases in smoking and duration of exposure. MN formation was not only seen in target tissues (exfoliated epithelial cells from the upper respiratory tract) but also in surrogate tissue (i.e. peripheral blood cells). Demircigil et al. (2010) found that workers (n = 50) from different workplaces exposed to crystalline silica-containing dust have more pronounced induction of MN in nasal cells (2.9-fold) and in lymphocytes (2.2-fold). These findings indicate that the part of genotoxic effect is caused by indirect mechanisms, particularly the formation of reactive oxygen species and inflammatory reactions (Borm et al., 2018; Fubini and Hubbard, 2003).

Consisted with our study, DNA damage in the lymphocytes and MN frequencies in buccal exfoliated cells were higher in workers (n:45) exposed to silica from mines and stone quarries. (Halder and De, 2012). Anlar et al. (2018) reported the possible genotoxic damage in ceramic workers exposed to complex mixture of chemicals mainly crystalline silica. Consistent with our findings, DNA damage in the lymphocytes, MN frequencies in buccal epithelial cells, and plasma 8-oxodG levels in the workers were higher when compared to controls. They concluded that occupational chemical mixture exposure in ceramic industry may cause genotoxic damage.

Our results show that the decreases in antioxidant enzyme activities in occupational marble dust exposure may decrease the protection against oxidative stress. Moreover, the increases in TNF-alfa and IL-1beta releases indicate the activation of immune response. It is concluded that the genotoxic effects of marble dust may be caused by indirect mechanisms, particularly the changes in inflammatory responses and oxidative stress.

There were some limitations of the present study. The crystalline silica content in the ambient air of marble dust-working areas could not be determined since only respirable dust measurements are obligatory in Turkey. Also, marble dust levels in different marble processing plants may vary and therefore the estimates from this cohort may not reflect all marble industries.

7. Conclusions

In conclusion, despite some limitations, our findings clearly confirm that the occupational exposure of silica-containing marble dust, may affect oxidative stress and immune parameters (SOD, CAT, GR, GPx enzyme activities; GSH, MDA, 8-oxodG, TNF-alpha, IL-1beta, CC16 levels) and leads to increases in DNA damage. It seems that it is necessary to improve ventilation practices, and use suitable protective masks, as well as to reduce the limit values in order to reduce occupational exposure to silica-containing marble dust.

Author contribution

Merve Becit: Conceptualization, Methodology, Software, Formal analysis, Investigation, Writing. Şule Çilekar: Methodology, Formal analysis. Mustafa Mert Başaran: Methodology, Software, Investigation, Writing- Reviewing and Editing. Halit Buğra Koca: Methodology, Writing- Reviewing and Editing. Sefa Çelik, Methodology, Writing-Reviewing and Editing. Sevtap Aydın Dilsiz, Conceptualization, Methodology, Software, Investigation, Resources, Writing- Reviewing and Editing, Project administration, Funding acquisition, Supervision

Specific information

Information on funding source.

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Ethics information

All volunteers were informed about the aim of our study and their

written consents were obtained. This study was approved by the ethics committee of Hacettepe University, Ankara, Turkey (Date: October 22, 2019 and Registration number: GO 19/975). The study was conducted in accordance with the ethical standards of the 1964 Helsinki Declaration. The ethical approval was added.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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