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Heavy Metal Burden and Antioxidant Enzyme Response in Marble Factory Workers

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ABSTRACT

Purpose: This study aimed to evaluate the impact of occupational exposure to marble factory dust on blood concentrations of selected heavy metals and the status of antioxidant enzymes in workers. **Method:** One hundred marble factory workers and an equal number of matched controls were included. Blood levels of chromium(Cr),cadmium (Cd), lead (Pb) and Nickel (Ni) were determined, and activities of antioxidant enzymes were measured spectrophotometric ally. **Results:** Workers exhibited significantly elevated blood concentrations of Cr,Cd and Pb compared to controls ($p < 0.01$). Antioxidant enzyme activities were markedly altered, with reduced superoxide dismutase (SOD) and catalase (CAT) activities, along with increased lipid peroxidation levels, indicating oxidative stress. Duration of exposure and tobacco use further exacerbated these alterations ($p < 0.05$). **Conclusion:** Occupational exposure to marble dust is associated with heavy metal accumulation and impaired antioxidant defense, contributing to oxidative stress in exposed workers. These findings highlight the need for monitoring heavy metal exposure and implementing preventive strategies in marble industries.

INTRODUCTION

Marble, a metamorphic rock with low silica content,(1) is primarily composed of calcite, dolomite, or serpentine. Its chemical composition includes 38-42% limestone, 20-25% silica, 2-4% alumina, 1.5-2.5% oxides, and 30-32% magnesium carbonate (2). Marble dust, produced during excavation, mining, and quarrying, accounts for 38-40% of global marble waste. (3). Long-term exposure to high dust density, temperature, and particulate material can cause DNA damage and occupational health issues. (4). Miners and building construction workers are those with the most exposure, and sand blasting, pre-claying, road construction, and the manufacture of ceramics carry risk of contact of the respiratory tract with CS particles (5). The dust from which workers who quarry, grind, polish and install marble are exposed includes particles of calcium carbonate and silica. It is well known that prolonged exposure to respirable crystalline silica can cause one of the oldest known industrial diseases, silicosis (6).

Numerous chemicals and hazards, including heavy metals, are present in the marble manufacturing process. Workers may experience health issues as a result of any of these risks. Previous research has shown that oxidative stress and genotoxic damage caused by heavy metal and other harmful chemical pollution are the causes of diseases, including cancer (3).

Numerous factors, such as exposure to industrial pollutants, chemical poisons, or other harmful radiation, can cause the body to produce free radicals (7). Any atom containing at least one unpaired electron in its outermost shell that exists independently is referred to as a free radical. These extremely reactive and unstable free radical entities stabilize by oxidizing membrane lipids, such as proteins' amino acids and nucleic acids' carbohydrates (8). Free radical species can be divided into two categories.

1) Reactive oxygen species 2) Reactive nitrogen species

Antioxidants are compounds that inhibit molecular oxygen consumption, protecting cells and organs against the toxicity of reactive oxygen species (ROS), including superoxide, hydroxyl radical, singlet oxygen, peroxyl radical, and peroxynitrite. (9). An excess of oxidants (free radicals or reactive oxygen/nitrogen species) or redox active metals that exceed the body's capacity to eliminate

or repair the ensuing damage in favor of redox active species is known as oxidative stress. Degradation of genetic, metabolic, and cellular alterations results from this. Antioxidant enzymes like glutathione reductase (GR), glutathione peroxidase (GPx), catalase (CAT), and superoxide dismutase (SOD) as well as antioxidants like glutathione (GSH) are useful indicators of oxidative stress (10).

The enzymatic defense systems in cells depend on a number of kinds of GST enzymes, referred to as glutathione S-transferases for several years, and this is the source of the commonly used acronym, GST which neutralize reactive species and help remove toxic chemicals. This vast collection of enzymes known as cytosolic GSTs are involved in the bio activation and detoxification of xenobiotic as well as the creation or degradation of several vital endogenous compounds (11). According to (12), they contribute to the regulation of a wide range of substances and compounds, including as dangerous materials, medications, carcinogens, oxidation products, and environmental contaminants.

Peshawar, the capital of Khyber Pakhtunkhwa province, is home to a significant marble industry that supports local livelihoods. The city's marble deposits are used for development, inner adornment, and handicrafts. However, workers in these small-scale factories are exposed to marble dust, highlighting the irony of Pakistan's industrialization's focus on production.

The present study's main goal was to perform a thorough investigation to look into the existence of oxidative stress enzymes and the heavy metal buildup, in marble industry workers.

MATERIALS AND METHODS

Study area and subject recruitment

A total of forty marble factories in District Peshawar, of Khyber Pakhtunkhwa Pakistan were chosen for the study. The study group included adult males (n=100 subjects and n=100 control) as they are more exposed to the marble dust. Every participant who took part in the study gave their written informed consent in form of questionnaire that included questions about age, education, smoking and other chronic health conditions.

I. Heavy metal analysis

The concentration of lead (Pb), chromium (Cr), cadmium (Cd), and nickel (Ni) in the serum samples were measured using Atomic Absorption Spectrometer at Peshawar University's Centralized Resource Laboratory. This study's procedure was based on(13) approach with few modifications. A 0.5 ml aliquot of each sample was combined with 5 ml of a freshly made, 3:1, v/v concentrated 69% HNO_3-37 % HCL combination, and the mixture was allowed to sit at room temperature for one hour. The mixture was cooked to 70 °C on a hot plate until it was clear. The filtrate was created up to 10 milliliters with distilled water after the solution was filtered via filter paper. The same procedure was used to run blanks, substituting 0.5 ml of distilled water for blood samples. Using an AA 40 FS Fast Sequential Atomic Absorption Spectrometer (Varian, AA240FS, USA), metal concentrations were measured. Various metal-specific lights with specific wavelengths were employed. For the elements being analyzed, a number of standards were run. Plotting the standard concentration versus absorbance allowed for the creation of the calibration curve. The concentrations were expressed in mg/l and measured in ppm.

II. Enzymatic evaluation

i. Catalase (CAT)

By measuring the drop-in absorbance brought on by H2O2 consumption, Aebi's approach was used to calculate cat activity (14) . Diluting plasma (25 µl of plasma with 2.5 ml of 50 mM phosphate buffer solution, pH 7) and 30 mM H2O2 were included in the reaction solution of CAT activities. For one minute, the Agilent 8453 spectrophotometer was used to measure the absorbance at 240 nm at 15-second intervals. In a similar manner, dis-tilled water was used to run blanks rather than plasma. The definition of one unit of CAT activity was a change in absorbance of 0.01 units per minute.

ii. Peroxidase Activity (POD)

POD activity was measured using Calberg and Mannervik's methodology(15). There were 0.1 milliliters of plasma, 0.1 milliliters of 20 mM guaiacol, 0.3 milliliters of 40 mM H_2O_2, and 2.5 milliliters of 50 mM phosphate buffer (pH 5.0) in the reaction solution. At 470 nm, the absorbance was measured one minute later. An absorbance change of 0.01 units/min was considered POD activity as per recommendations (16).

iii. Superoxide Dismutase (SOD) Activity

At 550 nm, SOD activity was determined by measuring the rate at which the superoxide radical (O2−) inhibited the reduction of cytochrome c. When xanthine and oxygen reacted, xanthine oxidase catalyzed the initial production of O_2. It was determined by the methodology of Christine (17)

RESULTS AND DISCUSSION

The detailed demographic factors have been explained in Table 1. The sample size each for the exposed and control subjects were taken as 100. A significant DNA damage ($p < 0.0001$) in the marble dust exposed subjects were observed (100. 17 ± 29. 81) than that of the control group (24. 44 ± 24. 47). Concentrations of heavy metals in blood is shown in the control and exposed individuals in Table 2 - 4. Levels of chromium [Cr], lead [Pb] and cadmium [Cd] were significantly higher in the workers exposed to heavy metals as compared to the controls ($p < 0.05$). Age relation with heavy metals have been shown in Table 3.

Enzymatic activity analysis between exposed group and control group significantly differs in CAT and POD enzyme levels. (Table 5-8) The control group for CAT had a mean of 6.25 ± 1.36 whereas the exposed group has significantly lower mean 5.11 ± 3.51 ($p = 0.013$). Furthermore, for POD, the mean in the control group was 8.21 ± 1.43, while the mean in the exposed group was significantly lower 6.55 ± 2.84 ($p = 0.001$). There was no significant difference between the control group (30.44 ± 1.62) and the exposed group (29.35 ± 2.61) for SOD with p greater than 0.05. Using an independent sample t-test (2-tailed), these

results show that occupational exposure causes a reduction in CAT and POD enzymatic activity while leaving SOD activity unchanged. Correlation is negative with respect to SOD and POD with increase in occupational exposure to marble waste enzymatic activity decreases.

Table 1. Characteristics of the subjects by exposure status (n = 100)

Variables	Control subjects (n=100)	Exposed subjects (n=100)	P value
Age (years, Mean±SD)	27.78±7.28	31.85±7.84	0.00
Education (Yes/No)	45/55	51/49	0.00
Gender (Male/Female)	Males	Males	0.00
Occupational exposures (years, mean)	0	7.23±4.73	0.00
Tobacco use (yes/no) %	0	41/59	0.00

Table 2. Concentration of heavy metals in the blood of exposed individuals

Heavy Metal	Control (Mean+SD)	Exposed	P -value
Cr	0.01±0.003	0.09±0.197*	0.00
Pb	0.32±0.043	1.46±0.35*	0.00
Cd	0.004±0.001	0.068±0.097*	0.00
Ni	0.385±0.0034	0.402±0.252	

*Statistically significant p value <0.05, independent sample T test 2 tailed

Table 3. Age relation with heavy metals

Heavy Metals	Below 30	Above 30
Cd	**0.94 ± 0.17**	**0.12 ± 0.22***
Pb	**1.56 ± 0.38**	**1.64 ± 0.37**
Cr	**0.62 ± 0.02**	**0.06 ± 0.02**
Ni	**0.41 ± 0.25**	**0.38 ± 0.24**

***Statistically significant p =0.035 which is <0.05**

Table 4. Heavy metals in smokers' vs nonsmokers marble workers

Heavy metals	Smokers	Non smokers
Cd	**0.07± 0.02**	**0.12± 0.25***
Pb	**1.68 ± 0.39**	**1.52± 0.39***
Cr	**0.67± 0.02**	**0.06± 0.03**
Ni	**0.35 ± 0.24**	**0.43± 0.25**

Statistically significant p<0.05 non parametric mann whiteny U test

Table 5. Anti-oxidant Enzymatic activity

Enzyme	Control N= 100 (Mean +SD)	Exposed N= 100 (Mean +SD)	p-value
CAT	6.25±1.36	5.11±3.51*	0.013
POD	8.21±1.43	6.55±2.84*	0.001
SOD	30.44±1.62	29.35±2.61	Not sig

***Statistically significant, independent sample t test 2 tailed p<0.05**

Table 6. Impact of Age on Antioxidant enzymes

Age groups	Enzymes	Mean+SD
Below 30	CAT	5.20±2.80
	POD	6.96±2.40
	SOD	29.50±2.30
Above 30	CAT	6.22± 4.406
	POD	7.51± 2.76
	SOD	29.09± 3.09

Not significant independent sample t test

Table 7. Correlation between Occupational Exposure and Anti-Oxidant Enzymes

Enzymes	Pearson's correlation	sig
CAT	r = -.021	-
SOD	r = -.215	0.03*
POD	r = -.322	0.001*

*statistically significant p<0.05

Large amounts of waste produced in the marble industry can contain high level of heavy metals such as lead, nickel, arsenic, cadmium, copper, mercury and manganese (18). A research has shown that workers in the metal industry, especially those working in smelting and refining, had high concentration of metals such as cadmium (Cd), lead (Pb) and chromium (Cr) in their blood and urine (19, 20).

Levels of chromium [Cr], lead [Pb] and cadmium [Cd] were significantly higher in the workers exposed to heavy metals as compared to the controls ($p < 0.05$). However, organisms have naturally many antioxidant defenses against such oxidative environment, classical antioxidant enzymes such as catalase, glutathione peroxidase and superoxide dismutase, and ROS scavenger non-enzymatic such as β-carotene, vitamin C, vitamin E, and uric acid (21).

CONCLUSION

This study highlights that occupational exposure to marble dust is associated with elevated levels of heavy metals and significant disturbances in antioxidant enzyme activities, reflecting increased oxidative stress in workers. The findings suggest that chronic inhalation of marble dust poses measurable health risks by disrupting redox balance. Implementation of dust control measures, consistent use of protective equipment, and regular biomonitoring are essential to reduce exposure and safeguard worker health. Future research should explore long-term health outcomes and intervention effectiveness in this occupational group.

Authors contribution

Conceptualization, Data curation, Investigation, Methodology, M.I.; Writing—original draft, N.S.; Supervision, M.K.

Consent to participate

Participants were informed about the study and provided their voluntary consent.

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