Asian Journal of Chemistry Vol. 20, No. 3 (2008), 1815-1820

Effects of Cement Dust Exposure on Malonyldialdehyde Levels and Catalaze Activities in Red Blood Cells

NAIM NUR*, ABDÜLKERIM YILMAZ†, SERPIL ERSAN‡, NURAN GÜLER††, HALDUN SÜMER and SEVTAP BAKIR‡‡
Department of Public Health, Faculty of Medicine
Cumhuriyet University, Sivas 58140, Turkey
Fax: (90)(346)2191155; Tel: (90)(346)2191010/2125
E-mail: nnur@cumhuriyet.edu.tr

This study was designed to investigate the plasma oxidant and antioxidant status in both cement plant workers and control subjects. Malonyldialdehyde (MDA) levels and catalaze (CAT) activity were investigated for 42 cement plant workers and the results were compared to 44 control subjects. The MDA levels in erythrocytes were significantly higher in the cement plant workers than in the control group. The CAT activity in the erythrocytes of workers occupationally exposed to cement dust was significantly lower than in the erythrocytes of control group. Occupational exposure to cement dust increased MDA but decreased antioxidant levels in cement plant workers.

Key Words: Cement plant workers, Malonyldialdehyde, Catalaze.

INTRODUCTION

In recent years, much attention has been paid to the possible role of radicals and antioxidants in diseases and aging. The major sources of radicals are air pollution, dust and chemical exposure, smoking and ionizing radiation[1]. Molecules of primary importance to the cement industry[2,3] are CaO, SiO_2, Al_2O_3, SO_3 and Fe_2O_3. Inhalation and skin contact is the most important route of entry of these chemicals into the body to produce adverse effects[4,5].

†Department of Internal Medicine, Faculty of Medicine, Cumhuriyet University, Sivas 58140, Turkey.
‡Department of Chemistry, Faculty of Engineering, Cumhuriyet University, Sivas 58140, Turkey.
††Public Health Nursing, School of Nursing, Cumhuriyet University, Sivas 58140, Turkey.
‡‡Department of Biochemistry, Faculty of Medicine, Cumhuriyet University, Sivas 58140, Turkey.

Cement plant workers are frequently exposed to cement dust that contains calcium oxide and silicates as its major components. Cement dust is a gray powder with a diameter ranging from 0.05 to 5.00 μm and manufactured from contaminated clay and lime stone[6,7]. In an industrial setting, inhalation and skin contacts by the stimulation other environmental factors such as antioxidants cause to unexpected hazards in biological systems, tissues and organs[8,9]. Many articles concerning harmful effects of cement (silicates) dust has been reported to cause laryngeal cancer, dermatitis[10], gastrointestinal tumors[10,11], respiratory diseases[11] and impaired lung function[10-13] and also augments free radical production[13-18]. The mechanisms for these effects are not fully understood, but there may be a link with asbestos and especially with reactive oxygen species (ROS), because an increase in the level of ROS and in the level of endogenous antioxidant enzyme plays an important role in endothelial cell function[13-18].

Although several reports have been published on various aspects of health and illness in cement workers[10-13], no study has been made on lipid peroxidation and antioxidant enzymes in this group.

In the present study, it was aimed to find out plasma malondialdehyde (MDA) levels as an index of lipid peroxidation and catalaze activity (CAT) as an index of antioxidant status in workers of cement factory in Sivas province of Turkey.

EXPERIMENTAL

Subjects selected for this study were non-smokers, non-drinkers, free from chronic illness and normotensives. A total of 86 Sivas cement factory men workers were included in the study. 42 Cement workers (experimental group), who were in the profession for 10 years, ranging in age between 24 and 46 years. The control groups (from the same area) consisted of 44 volunteer office men workers and ranging in age between 26-51 years. The written informed consent was obtained from all subjects before enrollment. Venous blood samples were collected from both control and cement workers after an overnight fast, as part of their routine monitoring.

Sample collection: Fasting venous 6 mL blood samples were collected from the median cubital vein into tubes without anticoagulants and 3 mL blood samples were drawn into tubes bearing 3.8 mmol/L ethylenediaminetetra acetic acid (EDTA). Use of the EDTA protects samples against oxidation and induction of hydroperoxide generation. Plasma was separated immediately. Erythrocytes were washed three times with an equal volume of isotonic saline (0.9 %), resuspended in an equal volume of distilled water and stored at -20 °C until analysis.

Biochemical analyses: Determination of erythrocyte concentrations of MDA was performed colorimetrically with small modifications from

the thiobarbituric acid reactive substances (TBARS) methods of Yagi[19] at 532 nm reported recently. In this method the blood samples were centrifuged at 4000 rpm for 10 min at 4 ºC to remove erythrocyte. The buffy coat on the erythrocyte sediment was used in the measurement assay of MDA levels. After each procedure, the erythrocyte-saline mixture was centrifuged at 4000 rpm for 10 min at 4 ºC. Erythrocyte sediments were resuspended four times with phosphate buffered saline (PBS) then MDA levels were expressed as $nmol^{-1}$ mL by the spectrophotometric analysis.

The CAT activity was also determined and compared in both experimental and control groups. The principle of assay is based on the determination of the rate constants of the hydrogen peroxide decomposition[20]. The decomposition of H_2O_2 can be followed directly by the decrease in absorbance at 240 nm. The difference in absorbance (ΔA_{240}) per unit time is a measure of CAT activity. CAT activity assay was as followed. The sample containing 2 mL. hemolysate and 1 mL. H_2O_2 at 20 ºC against a blank containing 1 mL, phosphate buffer instead of substrate and 2 mL, hemolysate. The reaction is started by addition of H_2O_2 and follow the decrease in absorbance with a recorder for about 15 s. The rate constant; k: $(2.3/\Delta t)$ (a/b) log (A_1/A_2). In this formula A_1 and A_2 are the absorbance values of hydrogen peroxide at t_1 (0 s) and t_2 (15 s) times, respectively, a is the dilution factor and b is the protein content of erythrocyte. CAT activity was expressed as k/g Hb.

The serum concentrations of alanine aminotransferase (ALT), aspartate aminotransferase (AST), total biluribin, cholesterol, triglyceride and uric acid were assessed by means of standard laboratory methods.

Statistical analysis: The statistical analysis was performed with the SPSS 12.0 software. Data between the groups were compared by student's t-test and the results were expressed as mean ± standard deviation (SD). Difference at the $p < 0.05$ level was considered to be statistically significant.

RESULTS AND DISCUSSION

The MDA levels, CAT activity and some biochemical values of 42 cement plant workers (experimental group) and 44 officer subjects (control group) were compared in this present study. The mean age of cement workers was 37.4 ± 7.2, control group was 36.9 ± 7.3 and the mean expose time to dust cement was 14.7 ± 5.3 years.

The MDA, CAT and some biochemical features of all subjects, both experimental group and control group are shown in Table-1. The levels of ALT and AST in the experimental group were significantly higher than those of controls. No difference in levels of cholesterol, triglyceride, total bilirubin and uric acid between experimental and control subjects were found ($p > 0.05$).

TABLE-1
VALUE OF MDA AND CATALAZE LEVELS AND SOME BIOCHEMICAL PARAMETERS STUDIED IN BOTH CEMENT WORKERS AND CONTROL SUBJECTS

Parameters	Cement workers (n = 42) mean ± SD	Control subjects (n = 44) mean ± SD
TBARS (equivalent to MDA $nmol^{-1}$ mL)	384.70 ± 71.90	313.30 ± 60.20*
Catalaze (k/g.Hb)	0.99 ± 0.53	1.65 ± 1.03*
Red blood cells (RBC)	5.15 ± 0.60	4.51 ± 0.20*
Hemoglobin (%)	76.50 ± 1.60	84.30 ± 2.10
AST (U/I)	25.30 ± 5.50	16.10 ± 4.80
ALT (U/I)	28.30 ± 6.60	18.30 ± 5.20
Triglyceride (mg/dl)	99.60 ± 32.40	109.40 ± 29.80
Uric acid (mg/dl)	5.40 ± 1.10	5.30 ± 1.01
Total bilirubin (mg/dl)	0.53 ± 0.02	0.57 ± 0.01
Cholesterol (mg/dl)	155.60 ± 33.20	158.60 ± 34.60

*$p < 0.05$; Student's t test.

As shown in Table-1 significant increase ($p < 0.01$) was found in the MDA levels in red blood cells (RBC) of cement plant workers (384.7 ± 71.9 $nmol^{-1}$ mL) when compared to the control group (313.3 ± 60.2 $nmol^{-1}$ mL) statistically. On the other hand, a significant decrease was found in the CAT activity in RBC samples of experimental group (0.99 ± 0.53 k/g Hb) when compared to the control group (1.65 ± 1.03 k/g.Hb) statistically ($p < 0.01$).

Occupational exposure to silica containing cement has been reported to cause respiratory diseases and impaired lung function[11]. Acute or chronic exposure to quartz is associated with the provocation of an inflammatory response and triggers of extensive host defense mechanisms[21,22]. These inflammatory reactions result in the secretions of cytokines, eicosanoids, lytic enzymes, chemotactic factors and ROS. Inflammatory reactions are sustained in quartz exposed animals resulting in the continuum of secretions of cellular products, repeated phagocytosis of quartz and the enhanced generation of ROS[13]. This quartz induced potentiation of ROS results in increased MDA levels and decreased antioxidants.

Activities of MDA, a product of lipid peroxidation, were determined to be higher ($p < 0.05$) in cement-exposed workers compared with the control group (Table-1). In some articles concerning with MDA levels were determined to be much higher in cement-exposed workers[23,24]. Increases in the level of MDA and low activity of superoxide dismutase (SOD) within red cells may be due to the exposure of cement workers to cement, which may result in the elevated production of free radicals. In other words,

oxidative stress is a balance between free radical production and antioxidant activity and it is possible that the raised MDA may be due to decreased antioxidant activity[17,19,25].

In a study showed that plasma MDA levels in asbestos-exposed workers were significantly higher[26]. We found increased higher liver function tests in cement workers. The others[10,23] also found increased ALT and AST activities in cement workers.

CAT, catalyzes the decomposition of hydrogen peroxide. The present study shows a significant decrease in the catalaze activity (Table-1). This decrease (in the activity of CAT) could be due to increase in MDA which can cross link with amino group of protein to form intra and intermolecular cross links thereby inactivating several membrane bound enzymes[27].

A decrease in red blood cells (RBC) count and Hb level (Table-1) in cement workers are also reported. This decreased level of RBC and Hb may be due to chronic exposure to cement dust. Cement bears calcium hydroxide as its important constituent[11]. It has been reported that chronic exposure to calcium hydroxide causes a decrease in RBC count and Hb[28]. The present observations are in accordance with the above-reported results.

The results that presented in this study indicate that the cement workers are exposed to more oxidative stress as evidenced by increased concentration of MDA levels, lowered CAT activity within red blood cells. These findings and other previous studies show possible relationship between increased MDA level and health problems frequency in cement plant workers[27,29,30].

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(*Received*: 31 January 2007; *Accepted*: 30 October 2007) AJC-6047