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Increased occupational coal dust toxicity in blood of central heating system workers

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Abstract

Coal dust causes lung diseases in occupational exposure. Reactive oxygen species have been implicated in the pathogenesis of its toxicity. In this study, serum enzymes, lipid profile and other biochemical values with oxidant/antioxidant status in whole blood and serum of central heating system workers (CHSW; the persons responsible for heating the apartment with coal) were determined to reflect the cell injury. Blood samples were obtained from CHSW ($n = 25$) and healthy individuals ($n = 25$). All values were measured in whole blood and serum. ANOVA was used for the estimation of statistical data. In the group of CHSW, creatinine, ferritin, alanin aminotransferase, aspartate aminotransferase, creatine phosphokinase, gamma glutamyl transferase, lactate dehydrogenase and glutathione reductase activities as well as triglyceride, very low density lipoprotein, protein carbonyl and malondialdehide were significantly higher, while transferrin, high density lipoprotein and catalase (CAT) activities were lower than the group of healthy individuals. This result is consistent with hypothesis that respirable coal dust generates lipid and protein oxidation and induces leakage of serum enzymes by cell damage. It also leads to imbalance in antioxidant defense system, lipid profile and other biochemical parameters.

Keywords

Central heating system workers, antioxidant, oxidant, vitamin, biochemical

Introduction

Occupational long-time exposure to dust particles induces an influx of inflammatory cells in the lung (Wallaert et al., 1990). Afterwards reactive oxygen species (ROS) consisting of OH^- radical, H_2O_2, HOCl and their common precursor O_2^- are released during the phagocytosis of inhaled dust particles (Malech and Gallin, 1987; Wallaert et al., 1990). These radicals might also be produced from the particles themselves (Berg et al., 1993; Petit and Boettner, 1990). It has been estimated that coal particles cause damage by their metal constituents (Dye et al., 1997; Ghio et al., 1997; Kodavanti et al., 1998; Pritchard et al., 1996).

Oxidative stress has been determined in coal miners and other workers in dusty jobs (Schins and Borm, 1999). Cellular damage was seen to be associated with ROS that was occurred by the exposure of coal dust. Generally, it is thought that coal dust forms ROS in two different ways. In the first way, ROS is generated by intrinsic properties of particles and iron content of coal dust. In the second way, the intensive formation of ROS occur by the oxidative burst of macrophages and neutrophils activated during phagocytosis and persistent inflammation (Bowden, 1987; Fubini and Hubbard, 2003).

Inhalation of coal dust may cause some lung and other organ diseases. Simple coal workers' pneumoconiosis, progressive massive fibrosis, chronic

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bronchitis, lung function loss and emphysema are more common among them (Schins et al., 1997). An enhanced antioxidant defense system occurs in the lung, interstitial fluid and (circulating) erythrocytes to eliminate ROS (Engelen et al., 1990).

Erythrocytes are circulating antioxidant carriers that leak into lung capillaries and have been shown to play a considerable role in protection against lung damage owing to ROS (Agar et al., 1986; Toth et al., 1984). Some changes are determined with respect to prooxidant/antioxidant status in blood of subjects exposed to long-term coal dust inhalation. This exposition usually give rise to increase of lipoperoxidation in plasma (Altin et al., 2004; Engelen et al., 1990; Schins et al., 1997) and decline in antioxidants such as reduced glutathione (Evelo et al., 1993) and antioxidant enzymes such as superoxide dismutase, glutathione peroxidase (Altin et al., 2004; Nadif et al., 1996, 2001; Schins et al., 1997) and glutathione s-transferase (Evelo et al., 1993).

By measuring blood antioxidants and products of oxidants, the role of oxidative stress in the grade of diseases in coal workers chronically exposed to coal dust particles can be designated. Recently, red blood cell antioxidant parameters were shown to decrease in some stages of coal workers' pneumoconiosis (Borm et al., 1990; Engelen et al., 1990). Erythrocyte glutathione s-transferase activity and glutathione concentration were decreased in the early stages of pneumoconiosis (Evelo et al., 1993).

The continuous production of ROS may damage antioxidant defenses and result in an oxidative stress leading to cellular injury in the lung (Sies, 1985). This damage causes denaturation of proteins, destruction of carbohydrates and peroxidation of lipids (Cantin and Crystal, 1985).

Coal dust exposure also affects some serum enzymes. For example, LDH activity increased with this exposure (Fan et al., 1994; Pinho et al., 2005). To comprehend early pulmonary responses after coal dust respiration might give rise to prevention and remedy procedures to encourage elimination and moderation of the development of irreversible occupational lung illnesses.

The purpose of the present study is to better understand the effect of occupational coal dust exposure on serum enzymes, lipid profile, some biochemical parameters and oxidant/antioxidant status of central heating system workers (CHSW; the persons responsible for heating the apartment with coal) by comparing them with the blood values of healthy adults.

Materials and methods

The study was conducted in Yuzuncu Yil University Medical Biology Department and Harran University Biology Department research laboratories during 2009. A written consent to participate in the study was obtained from the subject after they were thoroughly informed about the research details. Ethical approval for study was obtained from Harran University Faculty of Medicine Ethics Board. We divided all subjects into CHSW and control individuals. CHSW exposed to furnace coal dust particles while working on central heating system. The exposed group consisted of 25 male CHSW. The control group consisted of 25 males matched for age without a history of exposure to coal dust or other potentially toxic substances. The mean age of all subjects was 35 $\pm$ 5 years. The average working life of CHSW was 10 $\pm$ 1 years. CHSW group worked 7 months/year from October to April.

Totally 7 mL sample of venous blood was taken from each worker in the morning, before breakfast; 2 mL of it was taken in tube with EDTA, and the rest in biochemical tube. Samples were kept in a cool box at $+4^{\circ}$C until they were transferred immediately to the laboratory, where they were centrifuged. Serum was stored at -20°C until analysis.

Biochemical/serum enzymes/lipid profile analysis

The serum samples were obtained by centrifuging blood samples at 3000 rpm for 15 min at $+4^{\circ}$C. Serum enzymes (alkaline phosphatase (ALP; EC 3.1.3.1), alanin aminotransferase (ALT; E.C 2.6.1.2), aspartate aminotransferase (AST; EC 2.6.1.1), amylase (AMY; EC 3.2.1.1), creatine kinase (CK; EC 2.7.3.2), gamma glutamyl transferase (GGT; EC 2.3.2.2), lactate dehydrogenase (LDH; EC 1.1.1.27)), lipid profile (triglyceride, cholesterol, high density lipoprotein [HDL], low density lipoprotein [LDL], very low density lipoprotein [VLDL]) and other biochemical parameters (blood urea nitrogen [BUN], creatinine [CRE], ferritin [FER], glucose [GLU], magnesium [MG], phosphore [PHOS], total protein [TP], transferrin [TRSF] and urea [URE]) were measured in these serum samples by an autoanalyzer (Roche, Cobas Integra 400 plus).

Oxidant/antioxidant status analysis

Whole blood samples were hemolyzed with distillated water then they were centrifuged at 4000 *g* for 10 min at $+4^{\circ}$C. The clear upper supernatant fluid was taken.

Catalase (CAT), Glutathione Reductase (GR), Glutathione-S-Transferase (GST) activities and the levels of Reduced Glutathione (GSH), Malondialdehide (MDA), Protein Carbonyl (PC) and protein concentration were measured at this stage. β-carotene, vitamin A (retinol), C and E (α-tocopherol) concentrations were determined in serum.

CAT (EC 1.11.1.6) activity determination

The degradation speed of H_2O_2 by catalase was measured by reading the absorbance with a spectrophotometer set at 240 nm based on Aebi (1974) method.

GR (EC 1.6.4.2) activity determination

GR activity was assayed at 37°C and 340 nm by following the oxidized of NADPH by oxidized glutathione (GSSG) (Beutler, 1984).

GST (EC 2.5.1.18) activity determination

GST activity was assayed at 25°C spectrophotometrically by following the conjugation of glutathione with 1-chloro-2,4-dinitrobenzene (CDNB) at 340 nm as described by Mannervik and Guthenberg (1981).

GSH determination

Whole blood GSH concentration was assayed by reacting with *O*-phthaldialdehyde (OPT, 10 mg/10 mL methanol) according to the modified method of Lee and Chung, (1999). An aliquot of the supernatant was allowed to react with OPT for 5 min. Pure reduced GSH was used as standard for calibration. GSH samples were measured by using spectrofluorimetry (excitation at 345 nm, emission at 425 nm).

Antioxidant vitamin determination

The levels of β-carotene at 425 nm and vitamin A (retinol) at 325 nm were detected after the reaction of serum: ethanol: hexane at the ratio of 1:1:3, respectively, by Suzuki and Katoh (1990). Serum vitamin C level was determined after derivatization with 2,4-dinitrophenylhydrazine by Omaye et al. (1979). Vitamin E (α-tocopherol) was analyzed colorimetrically with 2,4,6-tripridyl-s-triazin and $FeCl_3$ after the extraction with absolute ethanol and xylene by Martinek (1964).

MDA determination

The values of MDA were determined as nmol/mg of protein. To measure the amount of oxidative damage in whole blood, levels of MDA were analyzed spectrofluorimetrically for each blood sample, using the modified thiobarbituric acid-reactive substance (TBARS) method by Hegde et al. (2003). The clear upper supernatant fluid was collected after centrifugation at 4000 *g* for 10 min at +4°C. MDA was measured by spectrofluorimetry (Jasco 6000, USA), with excitation at 520 nm and emission at 555 nm. Calculations were performed by using a linear regression from tetraethoxypropane for the MDA standard curve.

Protein carbonyl determination

Cayman's Protein Carbonyl Assay Kit utilizing the 2,4-Dinitrophenylhydrazine (DNPH) reaction to measure the protein carbonyl content in hemolysate was used (Levine et al., 1990). The amount of produced protein-hydrozone is quantified spectrophotometrically at an absorbance 360 nm by 96-well plate reader (SpectraMax M5). Then the carbonyl content was standardized to protein concentration.

Protein determination

The protein content in the whole blood was measured by the method of Lowry et al. (1951) with bovine serum albumin as the standard.

Statistical analysis

The results were analyzed by using one-way ANOVA test. The level of statistical significance was set at $p \leq 0.05$. Results were presented as means $\pm$ standard deviation (SD).

Results

Serum enzyme activities are given in Table 1. All enzyme activities were increased in the CHSW compared with healthy control. Among them ALT, AST, CK, GGT and LDH showed a statistically significant increase ($p \leq 0.05$).

Results for serum lipid profile are shown in Table 2. In the CHSW group, while triglyceride and VLDL level significantly increased, HDL decreased ($p \leq 0.05$).

Results for oxidant/antioxidant status are shown in Table 3. In the CHSW group, while CAT activity and GSH level reduced significantly, GR activity and

Table 1. Serum enzyme activities of CHSW and healthy controls

Serum enzymes	Control (mean ± SD)	CHSW (mean ± SD)
ALP U/L	83.88 ± 22.11	94.00 ± 29.24
ALT U/L	18.23 ± 5.09	25.00 ± 8.93[a]
AMY U/L	68.76 ± 19.51	70.23 ± 21.06
AST U/L	18.59 ± 4.42	26.86 ± 7.69[a]
CK U/L	85.79 ± 25.52	140.30 ± 41.69[a]
GGT U/L	16.53 ± 5.21	25.10 ± 7.95[a]
LDH U/L	342.00 ± 58.71	399.40 ± 68.05[a]

Abbreviation: ALP: alkaline phosphatase, ALT: alanin aminotransferase, AMY: amylase, AST: aspartate aminotransferase, CK: creatine kinase, GGT: gamma glutamyl transferase, LDH: lactate dehydrogenase, SD: standard deviation.
[a] Control versus treated $p \leq 0.05$ level.

Table 2. Serum lipid profile of CHSW and healthy controls

Serum enzymes	Control (mean ± SD)	CHSW (mean ± SD)
Triglyceride mg/dL	124.29 ± 37.34	199.93 ± 46.62[a]
Cholesterol mg/dL	169.10 ± 37.17	180.95 ± 43.85
HDL mg/dL	38.54 ± 8.11	30.64 ± 7.57[a]
LDL mg/dL	102.07 ± 22.65	106.68 ± 31.81
VLDL mg/dL	24.28 ± 7.78	42.33 ± 13.54[a]

Abbreviations: CHSW: central heating system workers, HDL: high density lipoprotein, LDL: low density lipoprotein, SD: standard deviation, VLDL: very low density lipoprotein.
[a] Control versus treated $p \leq 0.05$ level.

Table 3. Oxidant/antioxidant status in CHSW and healthy controls

Oxidant/antioxidant	Control (mean ± SD)	CHSW (mean ± SD)
CAT U/ mg protein	682.69 ± 32.11	190 ± 63.97[a]
GR U/ mg protein	0.045 ± 0.06	0.088 ± 0.016[a]
GST U/ mg protein	0.62 ± 0.08	0.49 ± 0.12
GSH nmol/ mg protein	10.64 ± 2.04	8.25 ± 1.62[a]
MDA nmol/ mg protein	3.21 ± 0.55	4.42 ± 1.03[a]
PC nmol/mg protein	4.85 ± 1.68	8.02 ± 3.20[a]
β-carotene μg/dL	351.55 ± 51.17	343.60 ± 105.59
Vitamin A μg/dL	638.59 ± 64.82	672.44 ± 152.71
Vitamin C mg/dL	0.066 ± 0.0032	0.067 ± 0.014
Vitamin E mg/d	2.21 ± 0.77	2.30 ± 0.72

Abbreviations: CHSW: central heating system workers, SD: standard deviation, CAT: catalase, GR: Glutathione Reductase, GST: Glutathione-S-Transferase, GSH: Reduced Glutathione, MDA: Malondialdehide, PC: Protein Carbonyl.
[a] Control versus treated $p \leq 0.05$ level.

MDA as well as PC levels increased compared with healthy control group ($p \leq 0.05$).

As shown in Table 4, serum CRE and FER levels significantly elevated but TRSF level reduced in the CHSW group ($p \leq 0.05$).

Discussion

Our study was designed to explore what role coal dust play in the changes of serum enzyme activities, lipid profile, oxidant/antioxidant status and other some biochemical values in CHSW. According to literary, this study is the first investigation conducted on CHSW blood values mentioned above. That is why, in this study we compared our data with previous studies published on coal miners.

We proposed that some people who are responsible for heating the apartment with coal might have insufficient accoutrements to combat the ROS that occur

Table 4. Serum biochemical values of CHSW and healthy controls

Biochemical values	Control (mean ± SD)	CHSW (mean ± SD)
BUN mg/dL	13.88 ± 3.69	14.93 ± 3.45
CRE mg/dL	0.61 ± 0.12	0.77 ± 0.09[a]
FER ng/mL	74.93 ± 23.32	129.41 ± 40.02[a]
GLU mg/dL	103.60 ± 22.26	97.73 ± 12.48
MG mg/dL	1.25 ± 0.16	1.32 ± 0.11
PHOS mg/dL	3.34 ± 0.80	3.68 ± 0.50
TP g/dL	7.48 ± 0.30	7.33 ± 0.56
TRSF g/L	2.92 ± 0.44	2.52 ± 0.35[a]
URE mg/dL	29.65 ± 7.94	31.87 ± 7.41

Abbrevaitions: BUN: blood urea nitrogen, CHSW: central heating system workers, CRE: creatinine, FER: ferritin, GLU: glucose, MG: magnesium, PHOS: phosphore, SD, standard deviation, TP: total protein, TRSF: transferring, URE: urea.
[a] Control versus treated $p \leq 0.05$ level.

after exposure to coal dust. We can adopt the hypothesis that antioxidant parameters in whole blood may reflect toxic risks to lung tissue.

In the current study, serum enzyme activities were higher in CHSW group than healthy controls. Among them ALT, AST, CK, GGT and LDH showed a statistically significant increase. The augmentation in activities of ALP and AMY were not significant. As lipid and protein oxidation were generated by ROS, elevations were observed in the amount of membrane-damaged cells (Lobner, 2000), and then the leakage of these serum enzymes into extracellular fluid had followed this process. Enhanced activity serum LDH observed in this investigation is consistent with the animal experiment realized by Pinho et al. (2005). They were determined high LDH activity in the fluid obtained from the bronchoalveolar lavage following coal instillation.

With respect to serum lipid profile, while serum triglyceride and VLDL levels significantly increased, HDL level decreased in the CHSW group as compared with healthy controls. But increases of cholesterol and LDL levels were not significant. Our data matches with the study of Armutcu et al. (2003); in that investigation they determined significantly increased serum total cholesterol, triglyceride, LDL and decreased level of HDL in coal miners compared to the controls. They reported that changes in lipid profiles of coal miners could be attributed to heavy and stressful working conditions and environmental factors. It can be concluded that coal dust may have a stimulating effect on the release of VLDL and triglyceride from liver and cause elevation in bloodstream.

In the CHSW group, while CAT activity and GSH level reduced significantly, GR activity and levels of MDA and PC increased compared with healthy controls. Decrease of GST activity and β-carotene levels as well as increase of vitamin A and vitamin C levels were not statistically significant in CHSW compared to controls. These findings may be considered as a result of exposition to respirable coal dust, which leading to production of ROS may be predictive of the inflammation and cell injury. The reduction of CAT activity may be related to inhibition of enzyme via ROS. Decrease in the activity of CAT arose from excessive production of superoxide anion because this anion inhibits CAT (Kono and Fridovich, 1982). This decrease in CAT activity is also determined in the study of Armutcu et al. (2007). According to us, GSH depletion is related to rather generation of ROS by the alveolar macrophages and neutrophils in the lung tissue. We can say that enhancement of GR activity in CHSW is due to increased GSH consumption. This enzyme allows the formation of GSH again by adding hydrogen to GSSG. Among the nonenzymatic antioxidants, it is well known that glutathione plays an important role in the defense mechanism of the lung (Zhang et al., 1999). In other studies, it was suggested that the cellular glutathione level may also be a source of oxidant protection against inflammation and the inhalation of mineral dusts, respectively (Smith et al., 1994; Zhang et al., 1999). Our result of GSH is in accordance with data of animal study conducted by Pinho et al. (2004). The rising levels of MDA and PC in CHSW may be related to oxidation of lipid and protein by ROS in cells. These results match with animal studies realized by Armutcu et al. (2007). In that study, they showed the increase of MDA levels in blood after exposition to inhalation of coal dust. The increase of PC level might be linked to progression of fibrosis in lung by coal dust particles. Pinho

et al. (2004) have demonstrated that acute exposure to coal dust could induce oxidative stress. This sustained response can be associated with lung inflammation and lung fibrosis. In addition, coal dust contains iron, which can stimulate hydroxyl radical formation, lipid peroxidation, and damages proteins, producing carbonylated proteins. Oxygen radicals can modify amino acid side-chains from protein aggregates, cleave peptide bands, and make proteins more susceptible to proteolytic degradation. In the process, some carboxyl residues are converted to carbonyl derivates (Halliwell and Gutteridge, 1999). No differences were observed in terms of serum β-carotene, vitamin A, C and E between control and CHSW groups.

Serum CRE and FER levels of CHSW significantly elevated but TRSF level reduced compared to controls. On the other hand there were no significant increases of BUN, MG, PHOS, URE and decreases of GLU and TP levels in CHSW when compared to control. Creatinine is chiefly secreted out of the blood by the kidneys. Measuring serum creatinine is the most commonly used indicator of renal function. Biochemically, renal damage is typically detected by an elevated serum creatinine level. In our study, increased level of creatinine in CHSW may indicate this damage. In humans, ferritin acts as a buffer against iron deficiency and iron overload. If ferritin is high there is iron in excess. It plays important roles in iron metabolism, storage and detoxification (Cook et al., 1974; Harrison and Arosio, 1996). However, the acute phase response induced by infection or systemic inflammation can elevate plasma ferritin concentration independent of the iron stores (Paracha et al., 2000; Tomkins, 2003; Witte, 1991). Transferrin is the primary iron-transport protein (Aisen et al., 1966). A low transferrin level may indicate excessive iron. For preventing formation of hydroxyl radicals, iron in the inflammatory site is withheld. Hence, iron is lost from the blood and iron accumulates in the liver, spleen and bone marrow. Therefore, our findings including high ferritin and low transferrin levels in serum of CHSW match with these mentioned phenomenon.

Results of this study led us to conclude that occupational exposure to coal dust give rise to harmful changes in blood of CHSW. Increased MDA and PC contents as well as imbalance in antioxidant defense system, lipid profile and other biochemical parameters might be indicators of these changes. Monitoring these values periodically is very important to determine the degree of biotoxicity. In addition, it is suggested that antioxidant supplement may be applied to CHSW.

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