

Some Antioxidant Enzymes in Workers Exposed to Pollutants

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ABSTRACT

The study was conducted in Mosul city to show the effect of pollutants on some antioxidant enzymes which included: catalase (CAT.), glutathione S-transferase (GST), superoxide dismutase (SOD) and glutathione peroxidase (GPx). The study included (153) persons represented in four groups, three of which were subjected directly to different pollutants (petroleum station workers (n= 37), workers in x-ray diagnosis (n=39) and cement production workers (n=33)). The fourth group as a control included outside city center living (n=44).

The results showed a significant decrease for CAT and GPx enzymes in all groups in comparison with control group. On the other hand, there was a significant decrease in GST with petroleum station and cement workers. For SOD, the results revealed a significant increase in workers of x-ray diagnosis, but a decrease non significantly in other groups. In addition, the increase of period of pollution, produced a decrease in CAT for all groups except in petroleum station workers and workers in x-ray diagnosis. Moreover a decrease in GST, SOD and GPx for all different pollutant groups were observed.

In conclusion, an increase exposure of different pollutants led to an increase in the oxidative stress in workers which decreased the antioxidant enzymes levels. This behaviors might give an indication for the oxidation that take place in exposed persons.

Keywords: Antioxidant enzymes, workers, pollutants

(SOD) (GST) -S (CAT) :
" (153) .(GPx)
(39) (37))

((33)

.(44)

GPx CAT

GST

SOD

CAT

SOD GST

GPx

INTRODUCTION

Antioxidant enzymes such as glutathione peroxidase, catalase, glutathione S-transferase, superoxide dismutase present in epithelial lining fluid (ELF) may protect the airways from oxidant injury induced by exposure to air pollutants (Kelly *et al.*, 1996; Banerjee, 2008). The antioxidants act as sacrificial substrates scavenging oxidant pollutants from the airways and thereby preventing oxidation of macromolecules such as lipids, proteins and carbohydrates.

Increased reduced glutathione (GSH) concentrations in the bronchoalveolar lavage (BAL) fluid have been reported in several cases thought to be mediated by oxidative stress, including cigarette smoking (Rahman and MacNee, 1996; Banerjee, 2008), adult respiratory distress syndrome and asthma. There is also considerable evidence linking chronic obstructive pulmonary disease with increased oxidative stress (Repine *et al.*, 1997).

The gaseous pollutants from combustion sources include a complex mixture of gases such as carbon monoxide (CO), nitric oxides (NO, NO₂), sulfur dioxide (SO₂) hydrocarbons, ash, transition metals and carbon particles (Miller *et al.*, 2009).

An accumulation of carbon monoxide (odorless, colorless gas) might result in a varied constellation of symptoms deriving from the compound's affinity for and combination with hemoglobin, forming carboxyhemoglobin (COHb) and disrupting oxygen transport. The elderly, the fetus, and persons with cardiovascular and pulmonary diseases were particularly sensitive to elevated CO levels (Raub and Grant, 1989; Regoli *et al.*, 2006).

Biological effects were observed when ionizing radiation strikes living tissues and damage molecules of cellular matter. Cellular function might be temporarily or permanently

impaired from the radiation, or the cell might be destroyed. Consequences might include degeneration or destruction of the irradiated tissues and the initiation of cancer (Stannard, 1990).

The physical and chemical nature of the particles in cement, their distribution in the respiratory tract and the biological events occurring in response to the particles determine their biological effects. Particle deposition in the respiratory tract depends on both particle size and the pattern of breathing (Soberanes *et al.*, 2009).

Recently, it was reported that airborne particles were associated with decreased in heart rate variability (Liao *et al.*, 1999 ; Gold *et al.*, 2000). Another report showed that nitrogen dioxide and PM_{2.5} (particulate matter with aerodynamic diameter less than 2.5 µm) were associated with defibrillator discharges due to ventricular arrhythmias in patients with implanted cardioverter defibrillators (Peters *et al.*, 2000 ; Banerjee, 2008).

The aim of the work is to obtain the effects of pollution (petroleum station workers, x-ray workers and cement workers) and the effect of pollution period on antioxidant enzymes (CAT, GST, SOD and GPx).

MATERIALS AND METHODS

The study included (159) persons represented in four groups, three of which were subjected directly to different pollutants (petroleum station workers, workers in x-ray diagnosis and cement production workers). The fourth group included outside city center living persons and considered as control.

For venipuncture, 10 ml sterile syringes equipped with (22G x 1.25) syringe needles were used and put in dry and clean plain tube. After coagulation, it was centrifuged at 4,000 x g for 15 minute. Serum was transferred into plain tube equipped with tight-fitting caps by disposable tips, then stored at -20 °C (Liang *et al.*, 1989). The spectrophotometer used type CE 1021 Ultra Violet and Visible, Cecil Instruments limited, England for all antioxidant enzymes measured.

Catalase activity (CAT) was measured from the decomposition of H₂O₂ (Mueller *et al.*, 1997). Briefly, 2 ml of diluted serum in 2 ml of phosphate buffer (50 mM K₂HPO₄ and 50 mM KH₂PO₄, pH 7.0) was transferred immediately to a cuvette containing 1 ml of 30 mM H₂O₂, and the change in absorbance at 240 nm was recorded for 1 min. One unit of catalase activity is defined as 1 mmol of H₂O₂ consumed per min per ml of serum.

The activity of glutathione S-transferase (GST) was determined spectrophotometrically at 340 nm by monitoring the rate of 1-chloro-2,4-dinitrobenzene(CDNB) conjugation with glutathione. The conjugation of (CDNB) with GSH proceeds by nucleophilic aromatic substitution of chlorine by thiol group of GSH, producing a dinitrophenyl thioether and chloride ion (Habig *et al.*,1974).

Superoxide dismutase activity(SOD) in serum was determined using a modified photochemical Nitroblue tetrazolum (NBT) method utilizing sodium cyanide as peroxide inhibitor with modification (Brown and Godstein, 1983).

Glutathione peroxidase (GPx) activity was measured by the method of Rotruck *et al.*, 1984. Briefly, the reaction mixture contained 0.2 ml 0.4 M sodium phosphate buffer, pH 7.0, 0.1 ml 10 mM sodium azide, 0.2 ml serum, 0.2 ml GSH, and 0.1 ml 0.2 mM hydrogen peroxide . The contents were incubate at 37°C for 10 min, the reaction was stopped with 0.4

ml 10% TCA and centrifuge. The supernatant was assayed for GSH content using Ellman reagent (19.8 mg DTNB in 100 ml 0.1% sodium citrate).

Body Mass Index (BMI) was determined using the formula: weight (kg)/height²(m²) (Al-Abbad and Al-Sowielem, 1998).

RESULTS AND DISCUSSION

Effects of pollutants on the antioxidant enzymes

The results of antioxidant enzymes for pollutant groups and control group were listed in Table (1).

The results in Table (1) showed that there were significantly decreased in all pollutants groups when compared with control group for CAT and GPx enzymes. On the other hand, there was a significant decreased in GST with petroleum station and cement workers. For SOD, the results revealed significant decrease in workers of x-ray diagnosis, but decreases non significant in other groups, which were similar to other reported results (Delfino *et al.*, 2009 ; Hatch, 2010 ; Vujovic *et al.*, 2010 ; Tsangaris *et al.*, 2010).

Table 1: The antioxidants enzymes of control and pollutants groups.

Parameters	Control group (n=44)		Petroleum station workers (n=37)		X-ray workers (n=39)		cement workers (n=33)	
	mean	SD	mean	SD	mean	SD	mean	SD
Age (year)	35.7	10.9	34.62	7.86	37.25	9.45	33.15	9.08
B.M.I (k.g./m ²)	26.71	2.45	26.93	2.45	26.45	2.19	24.15	2.13
CAT. (U/ml)	1.081	0.140	0.425*	0.089	0.54*	0.106	0.589*	0.091
GST(U/l)	5.29	0.160	3.38*	0.082	4.25	0.163	3.44*	0.075
SOD (U/ml)	1.401	0.06	1.065	0.034	1.118*	0.012	1.005	0.02
GPx (U/ml)	5.46	0.37	4.01*	0.26	3.87*	0.17	4.12*	0.27

*Different Significantly at P≤0.05.

High-dose exposure of gasoline from petroleum stations has been associated with a number of adverse health effects, including bone marrow depression and myelogenous leukemia in both rodents and humans (Hayes *et al.*, 2001). Although epidemiologic evidence does not permit reliable conclusions following human exposure to the low level of gasoline that typically is observed in environmental settings. Gasoline undergoes hepatic metabolism, generating hydroquinone, phenol and other compounds with the ability of redox cycling, which might cause excess generation of reactive oxygen species (ROS) (Bolton *et al.*, 2000). Therefore the antioxidant enzymes levels decreased to reduce the oxidative stress that might be produced from the ROS.

Particulate matter air pollution from cement induces the generation of ROS primarily from site III of the mitochondrial electron transport chain (Soberanes *et al.*, 2009). These antioxidant enzymes decreased because ROS increased. Beside of the increase used of

diesel-powered engines, particulate air pollution is increasingly being recognized as a major public health hazard and as a contributor to the burden of pulmonary and cardiovascular diseases (Soberanes *et al.*, 2009). Thus, there is a strong association between particulate air pollution and impaired lung function, deficits in lung function growth, worsening of asthmatic symptoms, and increased emergency room visits for asthma and chronic obstructive pulmonary disease (Atkinson *et al.*, 2001; McConnell *et al.*, 2003). The benzene combustions exposure was acting through inhibition of the nitric oxide (NO) pathway. In blood vessels, particularly conduit arteries, NO is a key dilator molecule and of considerable importance both in cardiovascular physiology and in pathophysiology (Miller *et al.*, 2009).

In addition, the skin is constantly exposed to environmental sources of ROS like ultraviolet (UV) light, ozone, and air pollution. To protect against oxidative damage the skin is equipped with a large network of enzymatic antioxidant defense systems, like catalase, superoxide dismutase (SOD), and glutathione peroxidase, and nonenzymatic antioxidants, like vitamin E, ascorbate, glutathione, and uric acid, which work in synergy to counterbalance oxidative stress (Hellemans *et al.*, 2003; Pandey and Rizvi, 2009; Nageshwar and Satish, 2010).

Effects of pollutant periods on the antioxidant enzymes

The results showed in Table (2) predicted that an increase of the period of pollution, produced a decrease in CAT for all groups except in petroleum station workers and workers in x-ray diagnosis. In addition a decrease in GST, SOD and GPx for all different pollutant groups were observed. Similar results were also published by other investigators (Ansari *et al.*, 2003 ; Erdal and Demirtas , 2010).

Epidemiological studies have found particulate air pollution in the urban environment to be associated with an increase in cancer, especially lung cancer (Pope *et al.*, 2002). Particles generated by combustion are composed of a carbon core to which compounds, such as metals and polyaromatic hydrocarbons (PAHs) (Han *et al.*, 2001).

One hypothesis to the observed adverse health effects is that particle matter (PM) can induce oxidative stress mediated by a particle-induced inflammation causing macrophages to release reactive oxygen species (ROS), by transition metals on the particle surface capable of generating ROS through Fenton reaction or by quinones in the particles that produce ROS through redox cycling (Li *et al.*, 1997; Han *et al.*, 2001).

More general responses were investigated as oxidative stress variations, including efficiency of antioxidant defenses (CAT, GST and GPx) scavenging capacity toward peroxy and hydroxyl radicals, onset of cellular damages (i.e., lysosomal destabilization), and loss of DNA integrity. On the other hand the investigations of marked accumulation of metals and PAHs in digestive tissues of organisms maintained in more petroleum station sites and increased ROS (Regoli *et al.*, 2006) .

Table 2: The different period of working for antioxidant enzymes in pollutants groups.

Antioxidant enzymes	Periods of working (Year)	Petroleum station workers (n=37)		X-ray workers (n=39)		cement workers (n=33)	
		mean	SD	mean	SD	Mean	SD
CAT(U/ml)	0-5	0.3197	0.055	0.372	0.001	0.361	0.071
	6-15	0.41	0.096	0.371	0.022	0.383	0.039
	16 and over	0.48	0.019	0.328	0.026	0.402	0.072
GST(U/l)	0-5	0.331	0.074	0.392	0.052	0.248	0.035
	6-15	0.31	0.059	0.296	0.072	0.245	0.045
	16 and over	0.351	0.046	0.326	0.073	0.237	0.018
SOD(U/ml)	0-5	1.05	0.02	1.108	0.042	1.057	0.026
	6-15	1.051	0.04	1.169	0.023	1.052	0.031
	16 and over	1.07	0.028	1.101	0.029	1.058	0.005
GPx(U/ml)	0-5	3.61	0.25	3.77	0.42	4.4	0.26
	6-15	3.11	0.31	3.47	0.23	3.95	0.31
	16 and over	2.85	0.26	3.08	0.17	2.57	0.27

The result of this study might suggest that the decrease of antioxidant enzymes levels in workers exposed to pollutants might be a part of the total antioxidant status protecting tissues from the effects of free radicals .

An increasing number of evidences demonstrated the involvement of reactive oxygen species (ROS) in the production of tumors by PAHs (Wattenberg, 1980; Frenkel *et al.*,1988). These oxygen species might lead to the formation of oxidative DNA damage. DNA damage (adducts and strand breaks) represents an early, detectable and critical step in the chemical carcinogenesis process and thus, might serve as an internal dosimeter for carcinogens (Van Delft *et al.*,1998). The induction of oxidative stress has been suggested as a possible mechanism of non-genotoxic chemical carcinogenesis and has been shown to participate in all stages of the carcinogenesis process, namely initiation, promotion and progression (Pryor, 1997).

It has been suggested that reactive oxygen species (ROS) and reactive nitrogen species (RNS) are involved in aging, mutagenesis, and carcinogenesis (Halliwell and Cross, 1994; Wiseman and Halliwell,1996). ROS include superoxide, hydrogen peroxide, and hydroxyl radical. RNS include nitric oxide and its derivatives such as nitrogen dioxide and peroxy nitrite. Particles in traffic exhausts can produce a significant amount of ROS. The smaller particles in ambient aerosols have higher ROS contents (Hung and Wang, 2001). Fine particles from traffic exhausts also contain polycyclic aromatic hydrocarbons (PAHs) (Strickland and Kang, 1999; Lai *et al.*, 2003).

Recently, ROS have been recognized as important signaling molecules that control diverse signaling pathways involved in a variety of cellular responses such as programmed

cell death, pathogen defense, and hormone signaling (Foyer and Noctor, 2005; Kwak *et al.*, 2006; Torres *et al.*, 2006). In addition, oxidative stress causes dramatic inhibition of the tricarboxylic acid cycle and large sectors of amino acid metabolism followed by backing up of glycolysis and diversion of carbon into the oxidative pentose phosphate pathway (Baxter *et al.*, 2007). Therefore, organisms have developed efficient systems to keep ROS levels in check and repair damage from attack by ROS.

All cells in the body are exposed chronically to oxidants from both endogenous and exogenous sources but come equipped with an antioxidant defense system. Nutrients both water soluble and lipid soluble, comprise an important aspect of the antioxidant defense system with which humans have evolved. Antioxidant is formulated by doctors based on results acquired in applied clinical conditions (Hughes, 2000; Berg *et al.*, 2007).

Antioxidant enzymes GPx and CAT were sensitive responding to the different pollution scenarios, showing good correlation to the chemical characterization (Contardo-Jara *et al.*, 2009; Vidal-Liñán *et al.*, 2010).

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