Effect of Some Environmental Conditions on the Oxidative Stress and Transition Metals Status in Iraqi Subjects

Muhanad Mohammed Nori [*], Saad Abdul-Rehman Hussain [**], Nada Khadum Al- Tae'e [***], Hameed Mahmood Majeed [****]

ABSTRACT:

BACKGROUND:

It has long been recognized that exposure to various metals is highly toxic, producing a wide variety of illnesses, including cancer. The role of these metals in free radical formation and initiation of lipid peroxidation was extensively studied; where the free form of iron or copper can effectively be involved in a free radical generation.

OBJECTIVES:

To evaluate the effects of the some environmental conditions on the levels of the oxidative stress markers, malondialdehyde (MDA) and glutathione (GSH), and some of the transition metals, iron, copper and zinc, in he serum of Iraqi subjects who exposed to different environmental conditions.

METHODS:

Blood samples were taken from 137 healthy male adults who work in different types of jobs (25 farmers, 25 workers in gasoline, 25 workers in liquid propane gas, 25 workers in casting, 25 workers in paints and 12 workers in plastic and rubber materials). The serum levels of MDA, GSH, iron copper and zinc were estimated and compared in different groups.

RESULTS:

The result showed that exposure of healthy subjects to different occupational hazards produces significantly different changes in the oxidative stress markers as revealed by excessive production of the lipid peroxidation end product (MDA) and depletion of the soluble antioxidant (GSH); and impairment of the trace element status (copper and zinc).

CONCLUSION:

The extracellular defense mechanisms were severely affected by the insults of oxidative stress processes due to environmental pollution in some work places in Iraq.

KEY WARDS: Oxidative stress, Markers**,** Malondialdehyde, Glutathione.

INTRODUCTION:

Toxic environmental agents include a number of chemicals and pollutants from man sources including the atmosphere, drugs, work place, food and water supply and from radiation and infectious organisms. These agents may exert multiple effects including excessive production of reactive oxygen species (ROS) [(1)], enhanced plasma membrane lipid peroxidation [(2)], covalent binding of multiple reactive metabolites [(3)], depletion and/or alteration of natural antioxidants (glutathione and protein thiols) [(4)] and alterations of intracellular / extracellular transition metals homeostasis [(5)].

The relationship between the environment and the concept of oxidative stress was based on the scientific evidence that free radicals and oxidative stress are of critical importance in various biochemical aspects during the life, like diseases, adaptive changes and physiological homeostatic and regulatory mechanisms [(6)]. In these regards, most pathological changes resulted from the interaction of biomolecules and the environment. That is, biological macromolecules by themselves explain a very low percent of diseases, while the remainder can be attributed to external “environmental” factors that act in conjunction with both genetic and acquired susceptibility [(7)]. Thus, two major approaches in prevention of environmental hazards are very well known, establishment of strategies to help people to modify hazardous life styles and/or use of chemoprevention (e.g. antioxidants) and reduction of involuntary exposure to the toxic agents in the environment. It has long been recognized that exposure to various metals is highly toxic, producing a wide variety of illnesses, including cancer. The role of these metals in free radical

* Department of Biology, College of Science, Al-Mustansirya University, Baghdad, Iraq.

**Department of Pharmacology and Toxicology, College of Pharmacy, University of Baghdad, Baghdad. Iraq.

***Department of Community Medicine, Al-Kindy College of Medicine, University of Baghdad, Baghdad, Iraq.

****Department of Community Health, Baquba Institute of Technology, Diyala, Iraq.

formation and initiation of lipid peroxidation was extensively studied; where the free form of iron or copper can effectively involved in free radical generation. It is now recognized that numerous transition metals (e.g. nickel, cadmium, arsenic, cobalt, tin, vanadium, etc), in addition to iron and copper, can also promote free radical formation and lipid peroxidation, presumably by the same or similar mechanism [(8)]. Since the role of free radicals-induced damage to the biological systems became an established fact in the explanation of the hazardous consequences of environmental exposure; this study was designed to evaluate the effects of various environmental conditions on the oxidative stress status and the levels of iron. Copper and zinc in Iraqi peoples.

SUBJECTS AND METHODS:

This study was carried out on 137 healthy male adults with age mean of 35.6 years, who work in different types of jobs, in the District of Baghdad. Clinically, they did not present any pathological abnormalities and medically examined for this purpose, and the presence of any recognizable disorder was considered as exclusion criteria.

The selected subjects were allocated in to 6 groups according to the type of job, through which they exposed to a certain type of environmental hazard, as follow: Group (A), include 25 farmers with age mean of 34.1 years, who are residents of rural areas around Baghdad city, they did not practice any other profession except agricultural activity.

Group (B), include 25 workers with age mean of 36.3 years, who work in Gasoline stations for at least 2 years ago, and exposed to petroleum products not less that 8 hours/day. Group (C), include 25 workers with age mean of 33.4 years, who work in Liquid Propane Gas (LPG) filling units for at least 2 years ago, and exposed to LPG fumes for not less than 8 hours/day. Group (D), include 25 workers with age mean of 35 years, who work in Casting Workshops for at least 2 years, and exposed to metals and their fumes for not less than 8 hours/day. Group (E), include 25 workers with age mean of 34.4 years, who work in painting for at least 2 years ago, and exposed to liquid paints and their fumes for not less than 8 hours/day. Group (F), include 12 workers with age mean of 33.1 years, who work in professions dealt with plastic and rubber materials for at least 2 years ago, and exposed to the components of polyethylene chloride (PEC) and rubber for not less than 8 hours/day. Blood samples (10 ml) were collected from all subjects by vein puncture and divided as follow were taken into plain tube, in order to obtain serum after clotting at room temperature.

Serum samples obtained after centrifugation at 3000 rpm for 10 minutes were kept frozen if not worked immediately. Serum levels of the oxidative stress markers, malondialdehyde (MDA) and reduced glutathione (GSH), were estimated according to the methods of Buege and Aust (1978) [(9)] and Godin et al (1988) [(10)] respectively. Serum iron levels were measured according to the method of Ceriotti and Ceriotti (1980) [(11)], serum copper was analyzed using a ready-made kit for this purpose[(12)] and Serum Zinc level was measured spectrophotometrically utilizing a ready-made kit for this purpose[(13)]. Results obtained in the study were presented as means ± S.E. Statistical analysis of data was performed utilizing Students t-test (unpaired), Benferroni test and ANOVA.

The significance level for all tests was taken as P value <0.05.

RESULTS:

The results presented in table 1 clearly indicate that exposure of healthy subjects to different environmental conditions produces significantly different changes in the extent oxidative stress state, indirectly assessed by the rate of production of malondialdehyde. According to the type of insult, liquid propane gas (LPG) has shown to produce the highest level compared to others, while farmers, gasoline, casting and painting workers did not show significant difference in the extent of oxidative stress. Plastic workers show the lowest level concerning this parameter (Table 1).

Glutathione, the first line non-enzymatic antioxidant is found to be severely affected by the type environmental insult, especially when the chances of turnover synthesis were impaired.

The results presented in table 1 clearly showed that, LPG workers demonstrated the lowest GSH levels in the serum; while paints and gasoline exposure appeared to be the second challenge, where workers in this filed show comparable serum GSH levels, but significantly higher than those observed in LPG workers (82% and 62% respectively). Plastics and Casting workers were presented with higher serum GSH levels compared with previously indicated types of exposure, and their antioxidant states seems to be comparable in this respect ($P>0.05$). Table 1 also clearly demonstrated that farmers have the highest GSH levels in the serum compared with others ($P<0.05$), reflecting the highest antioxidant capacity in this respect. As shown in table 2, no significant differences were observed in serum levels of zinc and iron among all groups ($p>0.05$), while serum copper level showed significantly higher levels in farmers and plastic workers compared to others.

Table1: Effect of different environmental conditions on the serum levels of the oxidative stress parameters, malondialdehyde (MDA) and glutathione (GSH) in Iraqi people.

Subjects Groups	MDA µmol/L	GSH nmol/L
Farmers n = 25	1.46 ± 0.21 [a]	358.0 ± 13.0 [a]
Gasoline workers n = 25	1.57 ± 0.22 [a]	152.0 ± 10.0 [b]
LPG workers n = 25	1.90 ± 0.20 [b]	94.0 ± 3.0 [c]
Painting workers n = 25	1.38 ± 0.19 [a]	279.0 ± 8.0 [d]
Casting workers n = 25	1.47 ± 0.20 [a]	171.0 ± 6.0 [b]
Plastic Workers n =12	0.81 ± 0.11 [c]	284.0 ± 7.0 [d]

Values represent mean ± S.E; n = number of subjects; values with non-identical superscripts (a, b, c) are considered significantly different (P< 0.05).

Table 2 : Effects of different environmental conditions on the serum levels of zinc, copper and iron in Iraqi people.

Subjects Groups	Zinc (µg/dl)	Cpper(µg/dl)	Iron (µg/dl)	Zinc/Copper ratio
Farmers n=25	101.0 ± 9.4 [a]	97.4 ± 8.40 [a]	64.2 ± 4.0 [a]	1.03 ± 0.11 [a]
Gasoline worker n=25	113.0 ± 12.2 [a]	74.6 ± 6.4 [b]	70.5 ± 10.1 [a]	1.51 ± 0.21 [a]
LPG workers n=25	122.0 ± 8.7 [a]	75.45 ± 4.3 [b]	71.7 ± 10.6 [a]	1.61 ± 0.15 [a]
Casting workers n=25	123.0 ± 6.8 [a]	60.4 ± 8.3 [b]	77.0 ± 6.1 [a]	2.03 ± 0.1 [b]
Painting workers n=25	126.0 ± 17.8[a]	61.8 ± 8.5 [b]	78.1 ± 3.8 [a]	2.03 ± 0.2 [b]
Plastic Workers n=12	107.0 ± 8.7 [a]	90.6 ± 9.9 [a]	61.2 ± 8.1 [a]	1.16 ± 0.2 [a]

Values represent mean ± S.E; n = number of subjects; values with non-identical superscripts (a, b) are considered significantly different (P< 0.05).

DISCUSSION:

Excessive formation of free radicals results in an increase in the process of lipid peroxidation, as evidenced by elevated levels of malondialdehyde (MDA), the end product of lipid peroxidation, in plasma and tissues of exposed subjects [(14)].

The results obtained in this study clearly showed that exposure to different environmental conditions resulted in an elevated levels of plasma MDA; and these varied according to the type of exposure, where LPG workers presented with maximum values which are significantly different from others (P<0.05). Farmers were presented with the lowest levels of oxidative stress marker, indicating low level exposure to pollutants and their hazards compared to other occupational conditions.

Many authors reported the importance of exposure to many types of pollutants as a risk factor of oxidative stress; so elevated serum levels of MDA were reported in painters compared to controls [(15)]. Consequently, many parameters of oxidative stress like GSH, MDA and others became objective indices for workers in health surveillance; and the role of these indices in the intoxication mechanism still need to be clarified [(16)]. It has been very well known that oxidative stress and lipid peroxidation nowadays suspected to be a common mechanism in many pathological changes; a fact which may shed a light on the mechanistic bases of different types of cellular or tissue damage produced by toxins and lead to a disease state; e.g. neurotoxicity, hepatic damage, nephrotoxicity and cancer [(17, 18)].

In addition to the occupational hazards indicated in this study, life style and psychological factors are also very important factors to be considered with respect to severity of oxidative stress. Epidemiologic studies suggested that up to 75-80% of all cancers are associated with life-style, especially tobacco, alcohol, diet and psychological stress. With respect to dietary factors, 30% or more of all cancers are thought to be directly or indirectly related to nutrition (obesity, increased fat consumption, in adequate intake of fruits, grains, vegetables and antioxidant vitamins) [19, 20].

Thus, the abnormal conditions of Iraqi community, concerning dietary intake, various types of stress and non-healthy environment may be the reason behind the generalized elevation of oxidative stress parameter, MDA, in most of selected groups.Glutathione (GSH) is the major cellular antioxidant and is found in high concentrations in most mammalian cells [21], and participates non-enzymatically and enzymatically in the protection against toxic compounds. Perhaps one of its most important functions is in the protection against oxidative stress caused by free radicals, many of which are generated during normal metabolism, or due to exposure to exogenous insults [22].

Excessive production of free radicals in the biological system due to many reasons depletes cellular reserve of GSH which consequently lead to cellular damage. Accordingly, GSH content (extra or intracellular) can be used as a predictor for the severity of the oxidative insult and the possible increase in susceptibility of those with low GSH levels to various types of diseases and metabolic disturbances. In this study, even when the average GSH content of most extracellular fluids is very low (2 μmol/L) [23], all the studied groups are presented with plasma GSH levels many folds lower than the value indicated above.

The significant differences observed between the studied groups in this respect could be utilized for the assessment and ranking of the harmful effects of each type of life style and occupation practiced by those workers. Additionally, health status of each group can be frankly assessed according to GSH status (e.g. susceptibility to infection, chronic diseases and cancer). Glutathione can act as a co-substrate for the glutathione peroxidase enzyme, which is responsible for the detoxification of hydrogen peroxide and other hydroperoxides.

This effect protects against oxygen free radicals and prevents the peroxidation of lipid membrane polyunsaturated fatty acids [24].

Meanwhile many carcinogens are found to be effectively detoxified by direct or indirect involvement of glutathione [25]. In the data presented in table 1, the occupational hazard of exposure to LPG was found to be the greatest depletor of GSH, where absorption through the skin and inhalation of these products provided a relatively free access to large quantities of these toxins to the biological system; while farmers, as a result of limited exposure to pollutants and different life style, showed lowest GSH depletion with respect to others; even when plasma GSH was lower than corresponding average value indicated in the literatures; which may be due to improper dietary intake and/or exposure to undetectable pollutants. Iron and copper are known to catalyze lipid peroxidation in vitro, and recent epidemiological data suggest that, these metal ions might also be involved in the pathogenesis of cardiovascular diseases in human [26], where high stored copper and iron levels, associated with elevated ceruloplasmin and ferritin, is considered as a risk factor in this respect.

Additionally, copper is an essential part of the antioxidant enzymes systems and could have a cardioprotective effect. However, a high cardiovascular risk has been associated with high, as well as low plasma copper. The results presented in table 2 showed that there is no significant changes between workers groups in serum iron levels which is already within the normal range; while serum copper appeared to be significantly lowered in gasoline, LPG, casting and painting workers compared to farmers and plastic workers, this effect may disturb the activation of some antioxidant enzymes (SOD and CAT) and make the oxidative stress more evident.

Low consumption of dietary zinc, and low serum levels were associated with increased prevalence of many occupational diseases including hypertension [27]. In table 2, no significant differences were observed in serum zinc levels in all groups of workers indicating that the homeostatic regulatory mechanisms which control body contents of this essential trace metal are still effectively functioning. For an attempt to overcome the great discrepancies in using serum levels of zinc and copper in explaining and evaluation of their role in oxidative stress states, Zn/Cu ratio was used and seemed to give more reliable expression of the changes in these elements, since there is an interplay between the variations in the free form of each one of them with respect to the other.

CONCLUSION:

Oxidative stress is a common consequence of exposure to different types of environmental pollutants during work times in various types of professions in Iraq; within the limitation of research conditions, the available data presented in this study, hardly enable us to determine specific biomarker in this respect to compare between the quantitative role of each type of environmental insult on the antioxidant systems.

REFERENCES:

1. Ehhlat DH. Radical ideas. Science 1998; 279: 1002-1003.
2. Forber JL, Kyle ME, Coleman JB. Mechanisms of cell injury by activated oxygen species. Lab Invest 1990; 62: 670-679.
3. Venitt, S. Mechanisms of carcinogenesis and individual susceptibility to Cancer. Clin Chem 1994; 40: 1421-1425.
4. Comporti M. Glutathione depleting agents and lipid peroxidation. Chem Physics lipids 1987; 45: 143 – 169.
5. Hippel S, Elstner EF. Biological and Biochemical effects of air pollutants. In; free radicals and oxidative stress: environment, drugs and food additives. Rice – Evans C, Halliwell B, Lunt GG, (eds.). London; Portland press; 1995: 153-161.
6. Ames BN, Shigenaga MK. oxidants are a major contributor of aging. Ann N Y Acad Sci 1992; 663: 85-96.
7. Bobak M, Leon DA. Air pollution and infant mortality. Lancet 1992; 340: 1010-1014.
8. Rayan TP, Aust SD. The role of iron in oxygen-mediated toxicities. Crit Rev Toxicol 1992; 22: 119-141.
9. Buge, J A, Aust SD. Microsomal lipid peroxidation. Meth Enzymol 1978; 51: 302-307.
10. Godin DV, Wohaieb S A, Garnett ME, et al. Antioxidant enzyme alterations in experimental and clinical diabetes. Mol Cell Biochem 1998; 84: 223-231.
11. Ceriotti F, Ceriotti G. Colorometric method for determination of iron. Clin Chem 1980; 26: 327-329.
12. Rice EW. Principles and methods of clinical chemistry, springfield, IL, Chartes C. Thomas, 1960, p. 157-159.
13. Solomons NW. On the assessment of zinc and copper nutriture in man. Am J Clin Chem 1979; 25: 1487.
14. Baliga R, Ueda N, Shan SV, et al. Oxidant mechanisms in glomerular disease. Curr Nephrol 1997; 20: 135-151.
15. Karagozler AA, Mehmet N, Lu KB, et al. Effects of long term solvent exposure on blood cytokine levels and antioxidant enzyme activities in house painters. J Toxicol Environ Health 2002; 65(17): 1273.
16. Jian L, Hu D. Antioxidative stress response in workers exposed to carbon disulfide. Int Arch Occup Environ Health 2000; 73(7): 503-506.
17. Kuloglu M, Atmaca M, Tezcan E, et al. Antioxidant enzyme activities and MDA levels in patients with obsessive-compulsive disorder. Neuropsychology 2002; 46(1): 27-32.
18. Bakan E, Tysi S, Polat MF, et al. Nitric oxide levels and lipid peroxidation in plasma of patients with gastric ulcer. JPN J Clin Oncol 2002; 32(5): 162-166.
19. Statland BE. Nutrition and cancer. Clin Chem 1992; 38: 1587-1594.
20. Miller AB, Berrino F, Hill M, et al. Diet and the etiology of cancer. European J Cancer 1994; 30 A: 207-220.
21. Meister A. Strategies for increasing cellular glutathione In: Boithioles in Health and Disease. Packer L, Cadenas E, (eds.), Dekker, New Yourk, 1995: 165-188
22. Kuhn MA. Oxygen free radicals and antioxidants. AM J Nutr 2003; 103: 42-46.
23. Gutteridge JM. Lipid peroxidation and antioxidants as biomarkers of tissue damage. Clin Chem 1995; 4: 1819.
24. Ivanova E. Ivanov B. Mechanism of extracellular antioxidant defenses. Bulg Acad Sci 2000; 4:22-30.
25. Batel BP, Raval GN, Raval R M, et al. Serum GST and GSH-Px activity in head and neck cancer patients. Neoplasma 2002 ; 44(4): 260-264.
26. Salonen JT, Nyssonen K, Korpela H, et al. Role of iron and copper in oxidative stress-induced vascular damage. Circulation 1994; 36: 803-806.
27. Singh RB, Wander G S, Rastogi A, et al. serum zinc levels as a risk factor in hypertension. Cardiovasc Drug Ther 1998 ; 12: 347-351.