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Oxidative Stress, Antioxidant Status and DNA Damage in a Mercury Exposure Workers

Hasan F. Al-azzawie, Akram Umran and Nadhem H. Hyader
Department of Biotechnology, College of Science, University of Baghdad, Iraq

Abstract: The present study was designed to evaluate changes in peripheral markers of oxidative stress, oxidative DNA damage and some biochemical markers during chronic $HgCl_2$ intoxication and to examine how the workers respond chronically to this pollutant. Fifty (50) fasting male workers, age range 28-61 years exposed to mercury in a chloroalkali factory at Al-Furat company in Babylon governorate, range duration period 5-18 years and 30 controls matched for age, diet and other demographic characteristics except exposure to chemicals were selected. Lipid peroxidation marker Malondialdehyde (MDA), antioxidant status markers enzymatic Superoxide Dismutase (SOD), Glutathione Peroxidase (GPx), non enzymatic antioxidant markers, Glutathione (GSH), vitamin C, vitamin E and oxidative DNA damage by comet assay were determined. Blood Mercury levels were significantly higher in the workers G2 and G3 groups compared with controls group G1 ($p<0.01$). MDA levels were also significantly increased and positively correlated with the concentration of Hg in group 3 ($r = 0.76$, $p<0.01$), probably to match the body chemical burden, while levels of non enzymatic antioxidant GSH, vitamin C, E and enzymatic antioxidant SOD and GPx were in contrast significantly lower in both workers than in controls ($p<0.01$). The present study indicates that workers occupationally exposed to mercury, in the particular conditions of exposure of this collective evaluated, show clear evidence of genotoxic activity in their lymphocytes especially in G3 than G2 against healthy control group G1 and this genotoxic activity (DNA damage by comet assay) is correlated positively with the long period of exposure to mercury pollution. DNA damage in mercury-exposed individuals suggests that mercury overload induces an imbalance in the redox cycle.

Keywords: DNA damage, inorganic mercury, oxidative stress biomarkers

INTRODUCTION

Mercury (Hg) is a highly toxic metal that results in a variety of adverse neurological, renal, respiratory, immune, dermatological, reproductive and developmental disorders (Risher and Amler, 2005). Its wide industry related effects on human and animal biosystem have been well documented (WHO, 1991) and general exposure to this biologically-active chemical agent has been shown to be exacerbated through contaminated water and food (Magos and Clarkson, 2006). Nowadays, large populations worldwide are exposed to relatively low levels of Hg, especially via the use of pesticides in agriculture and of fluorescent light bulbs as well (El-Shenawy and Hassan, 2008). In this context, Hg exists in a wide variety of physical and chemical states, each of which has specific characteristics for target organs (Aleo *et al*., 2002; Ghosh and Sil, 2008). For example, exposure to Hg vapor as well as to organic Hg compounds specifically affects the central nervous system (Vahter *et al*., 2000), while kidneys, liver and gastrointestinal tract are mainly targeted by inorganic Hg compounds, such as mercuric chloride ($HgCl_2$) (Ghosh and Sil, 2008; Schurz *et al*., 2000). In this respect, multiple mechanisms have been proposed to explain the biological toxicity of $HgCl_2$ by investigating the biochemical fate of various Hg forms (Gutierrez *et al*., 2006). Indeed, the Hg^{2+} form has shown a great affinity for endogenous biomolecules-associated thiol (-SH) groups (Clarkson, 1997) and it is invariably found attached to SH-containing proteins, small-molecular weight peptides (such as glutathione) and amino acids (such as cysteine) (Perottoni *et al*., 2004a), leading to a profound deterioration of vital metabolic processes (Sener *et al*., 2003; Wiggers *et al*., 2008). Consequently, the oxidative stress was strongly suggested as one of the crucial mechanisms in Hg-induced pathological aspects (Clarkson, 1997; Perottoni *et al*., 2004a; Lund *et al*., 1993). However, biochemical parameters are still more indicative of early physiological changes following subchronic and chronic Hg exposure (Wadaan, 2009). Therefore, the toxicological assessment of the general health condition should take into account the biochemical modulations induced by this pollutant during the first stages of contamination.

The risk of chemical toxicity is recognized to be greatest in the rapidly industrializing and restructuring developing countries (WHO, 1992; Uzma *et al*., 2008). This is remarkably at variance with the developed countries with ample facilities to reduce over exposure

Corresponding Author: Hasan F. Al-Azzawie, Department of Biotechnology, College of Science, University of Baghdad, Iraq

and the toxic effects of chemicals. These risks are particularly enhanced in developing countries where there is often little information on the safe handling or transportation of chemicals in industry and agriculture. Accumulating evidence indicates that exposure to chemicals including the work place leads to generation of free radicals which if unaccompanied by available antioxidant leads to oxidative stress (Anetor and Adeniyi, 2001; Flora, 2005). Indeed oxidative stress is being increasingly recognized as a possible mechanism in the toxicity and carcinogenesis of various chemicals including heavy metals, organic and inorganic solvents, gases and acids encountered in the work place (Ramsey, 1992; Anetor, 1997). Demonstration of excess free radical formation and deficit (absolute\relative) in antioxidant bioavailability in occupationally exposed individuals may serve as an early biochemical indicator of a pathophysiological state. Owing to the high potential to damage vital biological systems Toxic Oxygen Species (TOS) have now been incriminated in aging and over a hundred diseases including cancer causation usually preceded by genome instability (Selin, 2009).

The main objective of this study was to investigate if prolonged exposure of workers, occupationally exposed to several chemicals induces oxidative stress and implications for genome instability. This has not been determined in iraqian in particular and most developing countries in general, where knowledge of the harmful effects of chemicals is poorly recognized and Micronutrient Deficiency Disorders (MDDs), the major basis of the antioxidant system and nutrigenomics are common.

MATERIALS AND METHODS

Participants and specimen collection: Venous blood samples were collected from (50) males workers from Al-furat Company in Babylon province that used mercury electrodes to produce chlorine and soda during the period November 2010-march 2011. And (30) age matched healthy individuals with no significant signs, or symptoms of disease, to be used as a control group. All participants gave consent and the study was approved by the authorized medical office and the local Ethics Committee.

Determination of mercury in blood sample: Total mercury (Hg) concentrations were determined in whole blood using a modification of the method described by Shinyashiki *et al*. (1998). The samples were heated with 7 mL nitric acid and perchloric acid (2:1 V/V) for 1 h at 230-250ºC on a hot plate (Gerhardt, Germany). After digestion, mercury concentrations were measured by reducing vaporization-spectrometry atomic absorption method, using Nippon Instruments Mercury Analyzer, Model RA-2 (Tokyo, Japan). Data are presented as total mercury ng/mL.

Determination of Malondialdehyde (MDA): Serum MDA levels were estimated by the method of Beuge (Buege and Aust, 1978) using Thiobarbituric Acid (TBA). The acid reacts with MDA to form a stable pink color with maximum absorption at 535 nm. According to this method, 375 mg of TBA was dissolved in 2 mL of 0.25 N (HCl), followed by 15 g of Trichloroacetic Acid (TCA) for a total volume of 100 mL. The solution was heated in a water bath at 508ºC to dissolve TBA properly. Then, 1 mL of serum was combined with 2 mL of TCA-TBA-HCl and mixed thoroughly. The solution was heated for15 min in a boiling water bath. After cooling, the flocculent precipitate was removed by centrifugation. Sample absorbance was then determined at 535 nm against a blank that contained all reagents except the serum sample. Serum MDA concentration was expressed as nmol/mL.

Determination of Superoxide Dismutase activity (SOD): Whole blood SOD levels were measured using a commercially available kit (Ransod; Randox Laboratories Ltd.). Xanthine and xanthine oxidase were used to generate superoxide radicals, which react with 2- (4-iodophenyl) -3- (4-nitrophenol) -5 phenyltetrazolium chloride (INT) to form a red formazan dye. Superoxide Dismutase activity was then measured by the degree of inhibition of this reaction. One unit of SOD causes a 50% inhibition of the rate of reduction of INT under the condition of the assay. The concentration of SOD was expressed as (U/mL).

Determination of Glutathione (GSH) content: Analysis of blood GSH concentration was performed with method described by Ellman (1959) and as modified by Ohkawa *et al*. (1979). In brief, 0.2 mL of whole blood was added to 1.8 mL of distilled water and incubated for 10 min at 37C for complete hemolysis. After hemolysis 3 mL of 4% sulphosalicylic acid was added and tubes were centrifuged at 2500xg for 15 min. Supernatant (0.2 mL) was mixed with 0.4 mL of 10 mM 5, 5'dithiobis- (2-nitrobenzoic acid) (DTNB) and 1 mL phosphate buffer (0.1 M, pH 7.4). At the end of 5 min absorbance of the yellow chromogen is measured at 412 nm and it is directly proportional to GSH concentration.

Determination of erythrocyte glutathione peroxidase activity: Whole blood GP_X levels were measured using a commercially available kit (Ransel; Randox Laboratories Ltd., UK) according to the method of Flohe and Gunzler (1984), where GP_X catalyses the oxidation of glutathione by Cumene hydroperoxide. In the presence of glutathione reductase and NADPH, the oxidized glutathione is immediately converted to its reduced form with a concomitant oxidation of NADPH to NADP+. The decrease in absorbance at 340 nm was expressed as units per liter.

Determination of erythrocyte Glutathione Reductase activity (GRx): GRx activity of erythrocytes was

measured with a commercial kit (Calbiochem 359962; Calbiochem-Nova Biochem). First, 200 µL of the diluted sample and 400 µL of 2.4 mmol/L GSSG buffer (dissolved in 125 mmol/L potassium phosphate buffer, pH 7.5, 2.5 mmol/L EDTA) were added to 400 µL of 0.55 mmol/LNADPH (dissolved in deionized water). The absorbance was measured at 340 nm every 1 min up to 5 min using the UV-V is spectrophotometer.

Determination of vitamin C: Vitamin C determined using ELISA kit which allows for the specific measurement of human Vitamin C concentrations in Serum or plasma. This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for Vitamin C has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any Vitamin C present is bound by the immobilized antibody. An enzyme-linked monoclonal antibody specific for Vitamin C is added to the wells. Following a wash to remove any unbound antibody-enzyme reagent, a substrate solution is added to the wells and color develops in proportion to the amount of Vitamin C bound in the initial step. The color development is stopped and the intensity of the color is measured. Determination of vitamin E was determined used an Elisa kit.

Determination of DNA damage by comet assay: Single cell gel electrophoresis or comet assay was done as the protocol of Lee *et al.* (2004). Briefly, lymphocytes cell suspension was prepared by mixing of 1 mL of whole blood with 1 mL of lymphocyte prep and 1 mL of 1x PBS, then centrifuged at 3000 rpm for 10 min. Three layers were formed, erythrocytes precipitant, plasma supernatant and an intermediate white thin layer of lymphocytes which was carefully drawn out and resuspended in 1xPBS and centrifuged for washing. The pelleted cells where suspended with 1xPBS and counted using haemocytometer chamber. Fifty µL of lymphocytes -PBS suspension was mixed with 500 µL of comet low melting agarose at 37ºC and quickly put onto a microscope slide. A cover glass was put slowly on the slide to make a uniform layer of agarose-cell mixture. This slide was put at 4°C to allow rapid solidification of gel. Slides were then immersed into cold lysing solution (2.5 M NaCl, 100 mMEDTA, 10 mMTris), 1% Triton X-100 and 10% DMSO were added freshly. After 1 h of lysis, slides were immersed in freshly prepared alkali unwinding solution (200 mM NaOH, 1 Mm EDTA) for 1 h, then slides were transferred to a horizontal electrophoresis unit containing running buffer (300 mM NaOH and 1 mM EDTA pH>13.0). Electrophoresis was conducted for 20 min at 1 V/cm. DNA staining was done with silver staining and comets were visualized in a LEICA GMBH, Germany fluorescent microscope.

Statistical analysis: One-way Analysis of Variance (ANOVA) was used for statistical evaluation in all experiments. Data are shown as mean±S.D. The value of $p<0.05$ was considered statistically significant.

Table 1: Host information of workers and healthy subjects

Group	N	Age range (y)	Hg level (ng/mL)	Duration of exposure to Hg	p-value
G1	30	36-65	3.10±0.78	None	
G2	23	28-61	8.20±1.12	<10 years	<0.050
G3	27	42-58	15.30±1.34	>10 years	<0.001

Table 2: Effect of mercury toxicity on lipid peroxidation marker, enzymatic and non antioxidant parameters in workers G2 and G3 and healthy G1 group

Parameter	Healthy non exposure group 1	Exposure group G2 <10 years	Exposure group G3 >10 years
Mercury (ng/mL)	3.10±0.78	8.20±1.12**	15.30±1.34**
MDA (µmole/L)	2.40±0.60	6.30±1.10**	7.40±1.20**
GSH (µmole/L)	15±1.30	10±1.10*	8.30±1.80**
SOD (U/mL)	490±20	400±23**	377±12**
GPx (U/mL)	450±12	380±16*	320±11**
Vitamin C (mg/dL)	0.96±0.11	0.83±0.15*	0.62±0.21*
Vitamin E (mg/dL)	7.80±1.20	6.10±1.40*	4.30±1.20**

*: $p<0.05$; **: $p<0.01$; (Mean±S.D.)

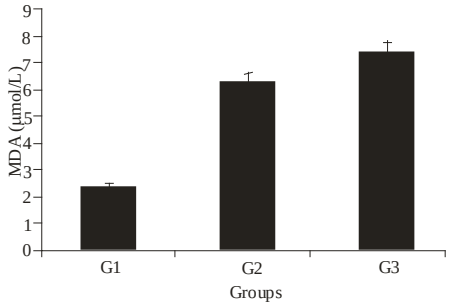

Fig. 1: Effect of mercury exposure on malondialdehyde levels in human workers groups (mean±S.D.)

RESULTS AND DISCUSSION

It is obvious from Table 1 that mercury concentration in the blood of the workers group G3 is about two fold than G2 compared with control group G1. Correlation is significant at the 0.05 level between the blood mercury concentration in the workers group and the period of their exposure to mercury while there is no correlation was observed between blood mercury concentration in the workers and their age.

Lipid peroxidation measurement: Lipid peroxidation products measures as Malondialdehyde (MDA) content were significantly increased in exposed workers G3 and G2 compared to the healthy control group G1 ($p<0.05$) as shown in Table 2 and Fig. 1 and a positive correlation was observed between the levels of Hg in blood of workers G3 and MDA levels as seen in Fig. 2.

MDA is one of the major oxidation products of peroxidized polyunsaturated fatty acids and increased MDA content is an important indicator of LPO. It has been shown previously that $HgCl_2$ increase MDA level in tissues (Mahboob *et al*., 2001; Park, 2007). Lipid

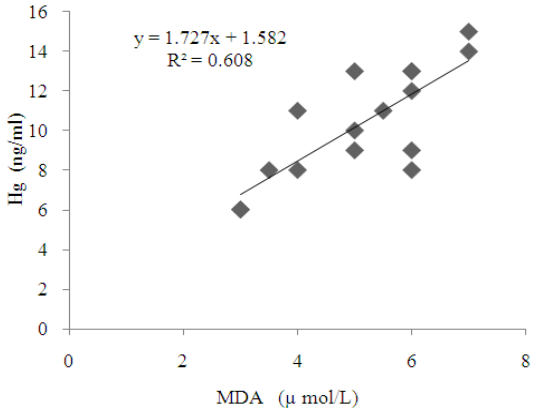

Fig. 2: Correlation between MDA levels and mercury concentration in workers group 3

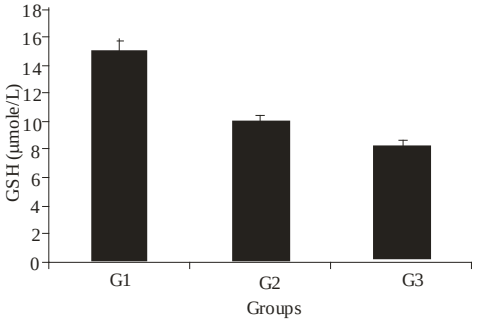

Fig. 3: Effect of mercury exposure on glutathione levels in human workers groups (mean±S.D.)

peroxidation is a chemical mechanism capable of disrupting the structure and function of the biological membranes that occurs as a result of free radical attack on lipids. The ability of mercury to produce ROS was indicated in the present study by increased amount of Lipid Peroxides (LPO).

Mercury causes cell membrane damage like lipid peroxidation which leads to the imbalance between synthesis and degradation of enzyme protein (Guzzi and Laporta, 2008). The excess production of ROS by mercury may be explained by its ability to produce alteration in mitochondria by blocking the permeability transition pore. Various mechanisms have been proposed to explain the biological toxicity of $HgCl_2$, including oxidative stress. The precise mechanism for ROS production by mercury is not known. Hg^{+2} reacts with thiol groups (-SH), thus depleting intracellular thiol, especially glutathione and causing cellular oxidative stress or predisposing cells to it (Gstraunthaler *et al*., 1983) and forming free radicals which may further increase lipid peroxidation. Molecular damage of the cells in mercury toxicity is by the formation of peroxyl radicals which can also be formed in lipid and non-lipid systems such as proteins (Schara *et al*., 2001). In this study, $HgCl_2$ initiate lipid peroxidation by generating free radicals and thereby interfering with the antioxidant system of the cell. This toxicity may be due to mercury-induced alterations in membrane integrity via the formation of reactive oxygen species and the perturbation of antioxidant defense mechanisms.

Mercury effect on reduced Glutathione levels (GSH): A significant reduction in glutathione levels in exposed workers G2 and G3 ($p<0.05$) compared with controls G1 was observed (Fig. 3) and a significantly negative correlation was observed between serum MDA content and a GSH level in workers exposed to mercury G3 ($r = -0.66$). Glutathione has been shown to be a significant factor in heavy metal mobilization and excretion, specifically with application to mercury, cadmium and arsenic. Glutathione depletion and glutathione supplementation have specific effects on mercury toxicity, both by altering antioxidant status in the body and by directly affecting excretion of mercury and other heavy metals in the bile. Mercury administration depleted GSH content and increase GSSG in liver that made hepatocytes more susceptible to oxidative damage, especially during increased free radical production. GSH is an important intracellular antioxidant that spontaneously neutralizes several electrophiles and reactive oxygen species, whereas GSH/GSSG ratio maintains the redox status of the cell. GSH is a substrate of enzyme GST, a phase II enzyme that plays a key role in cellular detoxification of xenobiotics, electrophiles and reactive oxygen species through their conjugation to GSH.

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Mercury effect on enzymatic antioxidant activity (SOD and GPx): A significant decrease in serum GPx and SOD activities in the exposure workers was detected especially in workers group G3 than G2 against control non exposure group G1 as seen in Fig. 4. Cells have a number of mechanisms to protect themselves from the toxic effect of reactive oxygen species. The SOD removes superoxide by converting it into H_2O_2 which is rapidly converted to water by

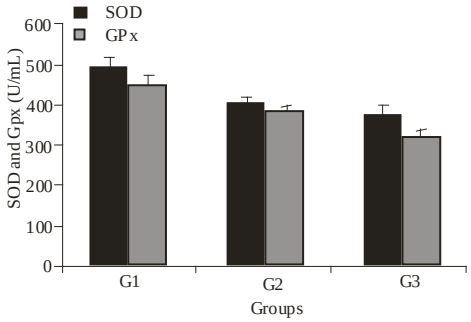


Fig. 4: Mercury effect on activities of enzymatic antioxidant (SOD and GPx) in human workers (mean±S.D.)

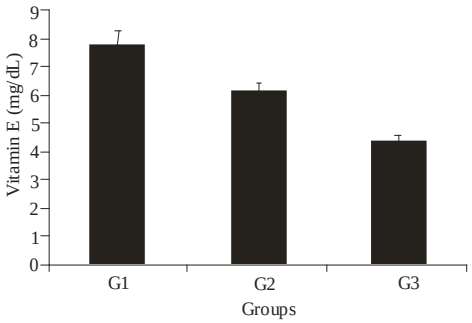


Fig. 5: Mercury effect on levels of vitamin E in human workers (mean±S.D.)

catalase. Therefore any alteration in the activity of these two enzymes may result in a number of deleterious effects due to accumulation of superoxide radicals and H_2O_2. So antioxidant intake could scavenge reactive free radicals that eventually lessen the oxidative tissue damage and subsequently improved the activities of these antioxidant enzymes.

Glutathione peroxidase is well known to defense against oxidative stress, which in turn needs glutathione as co factors. GPx catalyzes the oxidation of GSH to GSSG, this oxidation reaction occurs at the expense of H_2O_2. SOD is family of metallo enzyme, which is considered to be a stress protein which is synthesized in response to oxidative stress. It has been detected in a large number of tissues and organism and is present to protect the cell from damage caused by O_2 (Yu, 1994).

The scavenger role of antioxygenic enzymes in removing toxic electrophiles helps the cell to maintain its internal environment to a limited extent at lower concentration of mercury. An increase in the oxidative stress may be due to a decrease in the antioxidant defenses or due to an increase in the processes that produce oxidants (Hussain *et al*., 1999; Whaley-Connell *et al*., 2011). In this study, $HgCl_2$ initiate lipid peroxidation by generating free radicals and thereby interfering with the antioxidant system of the cell. This toxicity may be due to mercury-induced alterations in membrane integrity via the formation of reactive oxygen species and the perturbation of antioxidant defense mechanisms. Mercury also inhibits the activities of free radical quenching enzymes such as catalase, superoxide dismutase and glutathione peroxidase (Benov *et al*., 1990).

Mercury effect on vitamin E and C: The findings exhibited that mercury exposure reduced significantly ($p<0.05$) the levels of vitamin E in exposure workers Group (G3) and Group (G2) comparison with the control Group (G1) as shown in Fig. 5.

Vitamin E (α-tocopherol) is a fat-soluble vitamin known to be one of the most potent endogenous antioxidants. α-tocopherol is a term that encompasses a group of potent, lipid soluble, chain-breaking antioxidants that prevents the propagation of free radical reactions. Vitamin C is a water-soluble antioxidant occurring in the organism as an ascorbic anion. It also acts as a scavenger of free radicals and plays an important role in regeneration of α-tocopherol. Supplementation of ascorbic acid and α-tocopherol has been known to alter the extent of DNA damage by reducing tumor necrosis factor TNF-α level and inhibiting the activation of caspase cascade in arsenic intoxicated animals. These studies strongly believed that vitamins intake perspective, though observed in animal model, will have sustainable curative value among the already afflicted populations, neutralizing impact on freshly emerging metal poisoning and possible proactive protection to those potentially susceptible to heavy metal exposure. *In vivo* and *in vitro* antioxidant effect of vitamin-E on the oxidative effects of lead intoxication in rat erythrocytes suggests that simultaneous supplementation of vitamin-E to lead treated erythrocytes prevent the inhibition of δ-aminolevulinic dehydratase activity and lipid oxidation (Rendon-Ramirez *et al*., 2007).

Vitamin-E could be useful in order to protect membrane-lipids and, notably, to prevent protein oxidation produced by Hg intoxication. The protective action and the synergistic action of both vitamins (C and E) against lead-induced genotoxicity are discussed by Misra and Fridovich (1972). Early reports found that vitamin C might act as a possible chelator of lead, with similar potency to that of EDTA (Flora and Pachauri, 2010).

A significant decrease in levels of vitamin C in both exposure workers group G3 and G2 against control group ($p<0.05$) was observed (Fig. 6). This study demonstrates that these workers have increased oxidative stress. The significantly reduced ascorbate level in exposed workers compared to unexposed subjects, most probably reflects increased demand for this antioxidant to counter the excessive free radical burden. Vitamin C has generalized antioxidant property

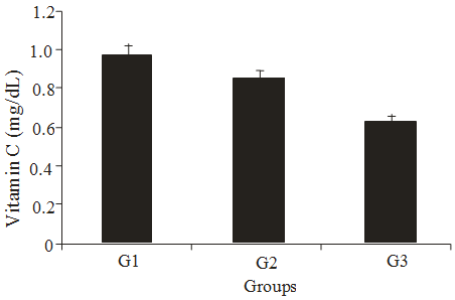
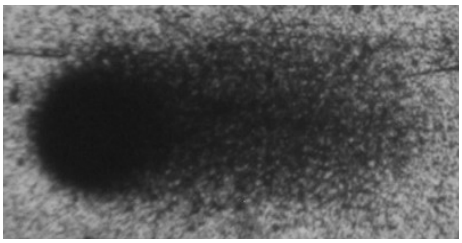
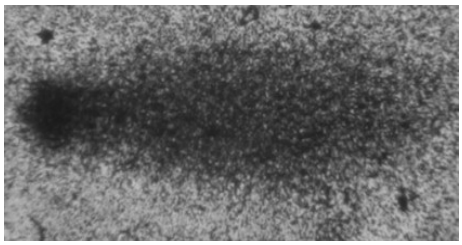


Fig. 6: Mercury effect on levels of vitamin C in human workers (mean±S.D.)

(A) Healthy non exposure



(B) Worker exposure to mercury for period <10 years



(C) Worker exposure to mercury for period >10 years

Fig. 7: A comet image of blood cells from healthy non exposure to mercury (A), worker exposure to mercury for period <10 years (B) and worker exposure to mercury for period >10 years (C). The extent of DNA damage was assessed by comet assay coupled with silver staining

and possesses preponderance in scavenging ability for the more common Toxic Oxygen Species (TOS) Thus, the demand for Vitamin C unlike limited or specific antioxidants will be high.

This significant reduction in vitamin C level probably confirms the suggestion that vitamin C is the first line of defense in the antioxidant defense System (Frei *et al*., 1989). The deficit in this potent antioxidant may increase risk of cancer. Vitamin C protects against DNA damage (Cail *et al*., 2001; Diatrich *et al*., 2003). DNA mutation in the absence of appropriate repair mechanisms may be the beginning of initiation phase of the carcinogenic process. On the other hand exposure of mercury in vivo has been shown to induce oxidative stress (Valko *et al*., 2006). Vitamin C (L-ascorbic acid) and Vitamin E (α-tocopherol) have a protective effect against mercury toxicity. Vitamin C is a strong antioxidant (Rao, 1997) having nucleophilic properties and binds to mercury ions (Hg^{+2}) to reduce mercury-induced damage. It further manifests its detoxification effect by removing or minimizing free radicals produced by mercury (Herbaczynska *et al*., 1995). Co-treatment with $HgCl_2$ and vitamin E was associated with a control rate of some disease, perhaps due to protection by vitamin E from oxidative tissue damage induced by mercury ion (Basu and Dickerson, 1996; Jha *et al*., 1995). Vitamin E prevents lipid peroxidation and maintains antioxidant enzyme activity and ascorbic acid levels in damaged tissue by inhibiting free radicals formation (Duval and Poelman, 1994). VC and VE inhibit oxidative damage in the liver and other tissues caused by mercury intoxication (Patil and Rao, 1999).

Our data is agree well with that reported that depletion of reduced glutathione, ascorbate and tocopherol on short term and long-term exposures were due to binding of mercury, even at low concentrations to the cellular antioxidant components (Kobal *et al*., 2004). In this study, at this concentration of $HgCl_2$, the vitamin C and vitamin E protected against $HgCl_2$-induced oxidative stress. These vitamins incline the enzymatic antioxidants levels to the control levels by effectively disposing the free radicals and possibly the mercury from the endogenous-SH groups. At concentrations that were similar to those found in plasma, VC and VE treatment, in combination, had no effect on MDA levels, or the levels of SOD, CAT and GPx activities in erythrocytes as compared to non-treated cells.

The mechanism by which plasma level of vitamins increased the levels of SOD, CAT and GPx activities in $HgCl_2$-treated cells most likely involved the dismutation of superoxide anions ($O^{2.-}$) and the decomposition of H_2O_2 and may represent an aspect of the cellular response to increased levels of ROS induced by $HgCl_2$ toxicity.

Effect of mercury on DNA damage: The comet assay is a simple, sensitive and rapid method that can be used to estimate DNA damage at the individual cell level through strand breaks, open repair sites, cross-links and alkali labile sites caused by oxidative stress (Tice *et al*.,

2000). The basis of the comet assay involves embedding a suspension of single cells in Low Melting Point (LMP) agarose on a microscope slide, thus creating a mini DNA agarose gel. The cells are lysed and then the DNA is electrophoresed. After silver-staining, the cells are observed under microscope and compared with control. The present study indicates that workers occupationally exposed to mercury, in the particular conditions of exposure of this collective evaluated, show clear evidence of genotoxic activity in their lymphocytes especially in group 3 than group 2 against healthy control group as shown in Fig. 7 and this genotoxic activity (DNA damage by comet assay) is correlated positively with the long period of exposure to mercury pollution. DNA damage in mercury-exposed individuals suggests that mercury overload induces an imbalance in the redox cycle. Enzymatic activities and thiol concentrations were strongly positively correlated with the mercury concentration.

On the other hand in lymphocyte cells from healthy non exposure subjects had tightly compressed DNA and maintained the circular form of the normal nucleus, with little or no evidence of comet formation (Fig. 7A). In contrast, cells from exposure workers to different doses of mercury displayed an altered appearance (Fig. 7B and C). The basis of this altered appearance, during alkaline unwinding (at pH 13.0) double stranded Supercoiled DNA was unwound and denatured.

As a result, double stranded complex DNA became single stranded and relaxed. So while electrophoresis was carried out, due to electric attraction, fragmentized DNA (negatively charged) migrated towards anode leaving the intact DNA in the head region. If nucleus contains more damaged DNA, large amount of these broken DNA (which are small in size compared to intact DNA molecule) will migrate in the tail region. The intensity of the comet tail relative to the head reflects the number of DNA breaks. Cells containing greater levels of DNA strand breakage cenerate comets with more intense ‘tails’ (Fig. 7C). Densitometric and geometric parameters of the comets as determined using image analysis software suggest that mercury period dose-dependently increased the comet tail. The alkaline comet assay showed a significant increase in comet length in all G3 and G2 groups as compared to controls G1.

In conclusion, even though the information available about the genotoxic effects of mercury exposure are contradictory, the present results show that workers occupationally exposed to mercury showed a significantly higher DNA damage frequency in peripheral blood lymphocytes. These observations support the need for preventive action that will improve conditions in the job environment and in micronutrient status since several studies have indicated that an increase in genotoxic effects is associated with an increased cancer risk.

REFERENCES

Aleo, M.F., F. Morandini, F. Bettoni, S. Tanganelli, A. Vezzola, R. Giuliani, N. Steimberg, J. Boniotti, B. Bertasi, N. Losio, P. Apostoli and G. Mazzoleni, 2002. In vitro study of the nephrotoxicity mechanism of mercuric chloride. Occup. Med. (Italian), 93(3): 267-278.

Anetor, J.I., 1997. An evaluation of the nutritional, metabolic and immune status in occupational lead toxicity. Ph.D. Thesis, University of Ibadan.

Anetor, J.I. and F.A.A. Adeniyi, 2001. Cadmium status in Nigerian cigarette smokers. Afr. J. Biomed. Res., 4: 23-26.

Basu, T.K. and J.W. Dickerson, 1996. Vitamins in Human Health and Disease. CAB International, Willingford.

Benov, L.C., I.C. Benchev and O.H. Monovich, 1990. Thiol antidotes effect on lipid peroxidation in mercury poisoned rats. Chem. Biol. Interact., 76: 321.

Buege, J.A. and S.D. Aust, 1978. The thiobarbuturic acid assay. Methods Enzymol, 52: 306-307.

Cail, L., J. Koropatrick and M.G. Cherian, 2001. Roles of vitamin C in radiation induced DNA damage in the presence and absence of copper. Chem. Biol. Interact., 137: 75-88.

Clarkson, T.W., 1997. The toxicology of mercury. Crit. Rev. Clin. Lab. Sci., 34: 369-403.

Diatrich, M., G. Block and N.L. Benowitch, 2003. Vitamin C supplementation decreases oxidative stress biomarker F2-isoprostane in plasma of non smokers exposed to environmental tobacco smoke. Nutr. Cancer, 45: 176-184.

Duval, C. and M.C. Poelman, 1994. Scavenger effect of vitamin E and derivatives on free radicals generated by photo irradiated phenomelanin. J. Pharm. Sci., 84: 107-110.

El-Shenawy, S.M.A. and N.S. Hassan, 2008. Comparative evaluation of the protective effect of selenium and garlic against liver and kidney damage induced by mercury chloride in the rats. Pharmacol. Rep., 60: 199-208.

Ellman, G.L., 1959. Tissue sulfhydryl groups. Arch Biochem. Biophy., 82: 70-77.

Flohe, L. and W.A. Gunzler, 1984. Assays of glutathione peroxidase. Meth Enzymol., 105: 114-421.

Flora, S.J., 2005. Metal induced oxidative stress and the role of antioxidants in the chellation therapy. Proceeding of SFRR International Conference. Bangalore, India, January 14 abstract.

Flora, S.J.S. and V. Pachauri, 2010. Chelation in metal intoxication. Inter. J. Environ. Res. Public Health, 7: 2745-2788.

Frei, B., C. England and B.N. Ames, 1989. Ascorbate is an outstanding antioxidant in human blood plasma. Proc. Natl. Acad. Sci. U.S.A., 83: 377.

Ghosh, A. and P.C. Sil, 2008. A protein from Cajanus indicus Spreng protects liver and kidney against mercuric chloride-induced oxidative stress. Biol. Pharm. Bull., 31(9): 1651-1658.

Gstraunthaler, G., W. Pfaller and P. Katanko, 1983. Glutathione depletion and in vitro lipid peroxidation in mercury or maleate induced acute renal failure. Biochem. Pharmacol., 32: 2969-2972.

Gutierrez, L.L.P., N.G. Mazzotti, A.S.R. Araújo, R.B. Klipel, T.R.G. Fernandes, S.F. Llesuy and A. Belló-Klein, 2006. Peripheral markers of oxidative stress in chronic mercuric chloride intoxication. Braz. J. Med. Biol. Res., 39: 767-772.

Guzzi, G.P. and C.A.A. Laporta, 2008. Molecular mechanisms triggered by mercury. Toxicology, 244: 1-12.

Herbaczynska, C.K., W.B. Ktoseiewicz, K. Cedro, W. Wasek, K.B. Panczenko and M. Wartanowicz, 1995. Supplementation with vitamins C and E suppresses leukocyte oxygen free radical production in patients with myocardial infarction. Eur. Heart. J., 16: 1044-1049.

Hussain, S., A. Atkinson, S.J. Thompson and A.T. Khan, 1999. Accumulation of mercury and its effect on antioxidant enzymes in brain, liver and kidneys of mice. J. Environ. Sci. Heal. B, 34(4): 645-660.

Jha, P., M. Flather, E. Lonn, M. Farkouth and S. Yusuf, 1995. The antioxidant vitamins and CVD disease: A critical review of epidemiologic and clinical trial data. Ann. Int. Med., 123: 360-372.

Kobal, A.B., M. Horvat, M. Prezelj, A.S. Briški, M. Krsnik, T. Dizdarevi, D. Mazej, I. Falnoga, V. Stibilj, N. Arneri, D. Kobal and J. Osredkar, 2004. The impact of long-term past exposure to elemental mercury on antioxidative capacity and lipid peroxidation in mercury miners. J. Trace Elem. Med. Biol., 17: 261-274.

Lee, E., E. Oh, J. Lee, D. Sul and J. Lee., 2004. Use of the tail moment of the lymphocytes to evaluate DNA damage in human biomonitoring studies. Toxicol. Sci., 81: 121-132.

Lund, B.O., M.D. Miller and J.S. Woods, 1993. Studies on Hg (II)-induced H_2O_2 formation and oxidative stress in vivo and in vitro in rat kidney mitochondria. Biochem. Pharmacol., 45: 2017-2024.

Magos, L. and T.W. Clarkson, 2006. Overview of the clinical toxicity of mercury. Ann. Clin. Biochem., 43: 257-268.

Mahboob, M., K.F. Shireen, A. Atkinson and A.T. Khan, 2001. Lipid peroxidation and antioxidant enzyme activity in different organs of mice exposed to low level of mercury. J. Environ. Sci. Heal. B., 36(5): 687-697.

Misra, H.P. and I. Fridovich, 1972. The role of superoxide anion in the autoxidation of epinephrine and a simple assay for superoxide dismutase. J. Biol. Chem., 247: 3170-3175.

Ohkawa, H., N. Ohishi and K. Yagi, 1979. Assay for lipid peroxides in animal tissues by TBA reaction. Anal. Biochem., 95: 351-358.

Park, K.E.J., 2007. Induction of reactive oxygen species and apoptosis in BEAS-2B cells by mercuric chloride. Toxicol. In Vitro, 21: 789-794.

Patil, G.R. and M.V. Rao, 1999. Role of ascorbic acid on mercuric chloride toxicity in vital organs of mice. Indian J. Environ. Toxicol., 9: 53-55.

Perottoni, J., L.P. Lobato, A. Silveira, J.B.T. Rocha and T. Emanuelli, 2004a. Effects of mercury and selenite on D-aminolevulinate dehydratase activity and on selected oxidative stress parameters in rats. Environ. Res., 95: 166-173.

Ramsey, 1992. Medical Management Guidelines for Acute Chemical Exposure. Agency for Toxic Substances and Disease Registry (ATSDR).

Rao, M.V., 1997. Mercury and its effects on mammalian systems a critical review. Indian J. Environ. Toxicol., 7: 31-37.

Rendon-Ramirez, A., J. Cerbon-Solorzano, M. Maldonado-Vega, M.A. Quintanar-Escorza and J.V. Calderon-Salinas, 2007. Vitamin-E reduces the oxidative damage on δ-aminolevulinic dehydratase induced by lead intoxication in rat erythrocytes. Toxicol. Vitro, 21: 1121-1126.

Risher, J.F. and S.N. Amler, 2005. Mercury exposure: Evaluation and intervention, the inappropriate use of chelating agents in diagnosis and treatment of putative mercury poisoning. Neurotoxicol., 26(4): 691-699.

Schara, M., M. Nemec, I. Falnoga, A.B. Kobal, M. Kveder and J. Svetek, 2001. The action of mercury on cell membranes. Cell Mol. Biol. Lett., 6: 299-306.

Schurz, F., M. Sabater-Vilar and J. Fink-Gremmels, 2000. Mutagenicity of mercury chloride and mechanisms of cellular defence: The role of metal-binding proteins. Mutagenesis, 15(6): 525-530.

Selin, N.E., 2009. Global biogeochemical cycling of mercury: A review. Annu. Rev. Environ. Resour., 34: 43-63.

Sener, G., A.O. Sehirli and G. Ayanoglu-Dülger, 2003. Melatonin protects against mercury (II)-induced oxidative tissue damage in rats. Pharmacol. Toxicol., 93(6): 290-296.

Shinyashiki, M., Y. Kumagai, H. Nakajima, J. Nagafune, S. Homma-Takeda, M. Sagai *et al*., 1998. Differential changes in rat brain nitric oxide synthase in vivo and *in vitro* by methyl merury. Brain Res., 798: 147-155.

Tice, R.R., E. Agurell, D. Anderson, B. Burlinson, A. Hartmann, H. Kobayashi, Y. Miyamae, E. Rojas, J.C. Ryu and Y.F. Sasaki, 2000. Single cell gel/comet assay: Guidelines for *in vitro* and in vivo genetic toxicology testing. Environ. Mol. Mutagen., 35: 206-221.

Uzma, N., B.M. Salar, B.S. Kumar, N. Aziz, M.A. David and V.D. Reddy, 2008. Impact of organic solvents and environmental pollutants on the physiological function in petrol filling workers. Int. J. Environ. Res. Public Health, 5(3): 139-146.

Vahter, M., A. Åkesson, B. Lind, U. Björs, A. Schütz and M. Berglund, 2000. Longitudinal study of methylmercury and inorganic mercury in blood and urine of pregnant and lactating women, as well as in umbilical cord blood. Environ. Res. Section A., 84: 186-194.

Valko, M., C.J. Rhodes, J. Moncol, M. Izakovic and M. Mazur, 2006. Free radicals, metals and antioxidants in oxidative stress-induced cancer. Chem. Biol. Interact., 160: 1-40.

Wadaan, M.A.M., 2009. Effect of mercury exposure on blood chemistry and liver histopathology of male rats. J. Pharmacol. Toxicol., 4(3): 126-131.

Whaley-Connell, A., P.A. McCullough and J.R. Sowers, 2011. The role of oxidative stress in the metabolic syndrome. Rev. Cardiovasc. Med., 12: 21-29.

WHO, 1991. World Health Organization. Inorganic Mercury, Environmental Health Criteria No 118, WHO, Geneva.

WHO, 1992. World Health Organization. Inorganic Mercury, Environmental Health Criteria No 118, WHO, Geneva.

Wiggers, G.A., F.M. Peçanha, A.M. Briones, J.V. Pérez-Girón, M. Miguel, D.V. Vassallo, V. Cachofeiro, M.J. Alonso and M. Salaices, 2008. Low mercury concentrations cause oxidative stress and endothelial dysfunction in conductance and resistance arteries. Am. J. Physiol. Heart Circ. Physiol., 295: 1033-1043.

Yu, B.P., 1994. Cellular defenses against damage from reactive oxygen species. Physiol. Rev., 74: 139-162.