

Research Article

Oxidative Stress in Workers Occupationally Exposed to Lead

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Abstract

Lead (Pb) is a pervasive and common environmental toxicant which has caused serious occupational diseases globally, and now a major public health challenge in Nigeria. The present study was designed to assess the status of blood lead levels (BLLs) and plasma markers of oxidative stress in individuals occupationally-exposed to lead. Thirty-eight lead exposed workers, comprising, eighteen (18) battery chargers (BC), ten (10) spray painters (SP) and 10 (ten) mechanics (MC) were recruited for this study after an informed consent. They were artisans (aged between 25 and 55 years) who have practiced their professions for a period of 17 to 25 years. The control group consisted of fifteen clinically healthy volunteers (CT). Blood Lead Levels (BLL) and antioxidant and markers of oxidative stress: superoxide dismutase (SOD), catalase (CAT), reduced glutathione (GSH), malondialdehyde (MDA), nitric oxide (NO), total antioxidant potential (TAP) and total plasma peroxides (TPP) were determined in them, using atomic absorption spectrophotometer and spectrophotometry methods respectively. Statistical analysis was done using the student's t-test and ANOVA. The BLLs increased significantly in only BC and SP but not in MC when compared with controls. Specifically, BLLs were of the order: BC (5.5 folds) > SP (4 folds) > MC (1.5 folds), although there was no significant difference between the blood lead levels in MC ($10.60 \pm 2.55 \mu\text{gPb/dl}$) and CT ($8.51 \pm 4.55 \mu\text{gPb/dl}$). The mean values of plasma MDA contents were significantly ($p < 0.05$) higher in all lead-exposed workers (BC, SP and MC) when compared with the controls (CT). The mean values of plasma Total Plasma Peroxide (TPP) and Oxidative Stress Index (OSI) were significantly ($p < 0.05$) increased in BC, SP and MC when compared with the controls (CT) while there were no significant ($p > 0.05$) changes in the mean values of NO in BC, SP and MC when compared with the controls (CT). The mean values of plasma antioxidant markers CAT, SOD and GSH were significantly ($p < 0.05$) reduced in BC, SP and MC when compared with the controls. These findings show that lead plays an active role in the generation of reactive oxygen species and oxidative stress in workers occupationally- exposed to lead and that adjuvant antioxidant therapy may be needed to avert the consequences of oxidative stress.

Keywords: Lead (Pb), Lead intoxication, Oxidative stress, Nigeria

INTRODUCTION

Lead is an environmentally persistent toxicant that has detrimental effects on physiological, biochemical and behavioral dysfunctions which have been documented in animals and humans (Ruff *et al.*, 1996). The metal affects the central and peripheral nervous systems, haemopoietic system (De Silva, 1981), cardiovascular system, kidneys, and reproductive systems (Vaziri *et al.*, 1999b). The mechanisms of lead-related pathologies include direct oxidant effects of lead on tissues and cellular components, as demonstrated in a number of animal studies (Vaziri *et al.*, 1999b). Lead toxicity leads to the generation of reactive oxygen species (ROS) and a direct depletion of antioxidant. Lead is shown to alter antioxidant activities by inhibiting functional SH groups in several enzymes such as Aminolevulinic acid dehydratase (ALAD), superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), and glucose-6-phosphate dehydrogenase (G6PD) [Hsu and Guo, 2002]. Erythrocytes are vulnerable to lipid peroxidation (LPO) due to their high content of polyunsaturated lipids (Puppo *et al.*, 1998). The erythrocyte has several cellular defence mechanisms to prevent the buildup of ROS and collectively protect it from

oxidative damage. Lead potentially induces oxidative stress and accumulated evidence support the role of oxidative stress in the pathophysiology of lead toxicity (Ercal *et al.*, 2000; Farmand *et al.*, 2005). Several epidemiological studies have reported that even at low levels, lead has a graded association with several ill health conditions including renal and cognitive impairment (Ekong *et al.*, 2006; Lee *et al.*, 2006 and Menke *et al.*, 2006). Recent studies also report positive association between lead exposure and oxidative stress markers (Menke *et al.*, 2006 and Muntener *et al.*, 2003). Although both moderate and subclinical effects of occupational lead poisoning are common in many countries of the world, occupational exposure is entirely unregulated in developing countries like Nigeria, India, etc. and a little monitoring has been conducted in developed countries [Verrula *et al.*, 1990]. In animal models, doses of lead exposure determine the levels of lead-induced oxidative stress in various target sites including lung, blood vessels, testes, sperm, liver, and brain (Puppo *et al.*, 1998).

Previous investigators did not consider effects of varied concentrations of lead on the status of oxidative stress in human beings. This present study focuses on the degree of lead-induced oxidative stress in three Nigerian professionals

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(battery chargers, spray painters and auto-mobile mechanics) with different levels of lead exposure.

MATERIALS AND METHODS

Human subjects

Thirty-eight lead exposed workers, including 18 battery chargers, 10 spray painters and 10 mechanics were recruited for this study. They were artisans who have practiced their professions for a period 10 to 25 years. Fifteen apparently healthy health workers of University College Hospital, Ibadan, Nigeria, served as controls. Five milliliters (ml) of venous blood sample was taken from the antecubital vein of every participant into a plain bottle and allowed to clot. After retraction, the serum was separated and stored at -20°C until ready for analysis. As a rule, subjects were not on any medication at the time of the study and had no previous history of serious cardiovascular, renal, hepatic, endocrine, metabolic or gastrointestinal disease. Ethical approval was obtained from the UCH/UI Ethical Committee on human research.

Reagents

Epinephrine, glutathione (GSH), 5, 50-dithiobis (2-nitrobenzoic acid), hydrogen peroxide (H₂O₂), thiobarbituric acid and 1-chloro-2, 4-dinitrobenzene was purchased from Sigma Chemical Co. (USA). All other reagents were of analytical grade and were obtained from the British Drug Houses (UK).

Methodology

The MDA, a product of lipid peroxidation was determined by using the method of Varshney and Kale (1990) and expressed as micromoles of MDA/ ml. Total Antioxidant Potential (TAP) was determined using the Ferric Reducing / Antioxidant Power (FRAP) assay (Harma *et al.*, 2003). The method of Harma *et al* (2003) was used for the determination of Total Peroxide Potential (TPP), (Harma *et al.*, 2003). The Oxidative Stress Index (OSI), an indicator of the degree of oxidative stress was determined as the percent ratio of the total plasma peroxide (µMol H₂O₂/L) to the total antioxidant activity (µmol.Trolox equiv. / L) (Harma *et al.*, 2003). NO was determined using the method described by Wanchu *et al* (Wanchu *et al.*, 2000). Superoxide dismutase (SOD) was assayed by the method described by Misra and Fridovich (1972), Catalase (CAT) was determined using the method described by Chiba *et al* (1996) and reduced GSH was determined at 412 nm using the method described by Beutler *et al.* (1963).

Statistical analysis

All statistical analyses were performed using Statistical Package for Social Sciences (SPSS) for windows, version 15.0 (SPSS Inc. Chicago, USA). The data were expressed as Mean ± SD. Student (t) test and ANOVA were used for the comparison of lead exposed workers and controls. Pearsonian correlation coefficient (r) was calculated. The changes were considered significant, when p-values were less than 0.05.

RESULTS

The mean blood lead levels increased significantly in only BC and SP but not in MC when compared with controls. The blood lead levels were of the order: BC (5.5 folds) > SP (4 folds) >

MC (1.5 folds) > controls (as shown in Table 1). The mean values of plasma MDA contents were significantly (p<0.05) higher in all lead-exposed workers (Table 2). Compared to the control subjects, the changes in the plasma LPO expressed as MDA accounted for 81.4%, 65.2% and 43.4% percentage increase in BC, SP and MC respectively. The mean values of plasma TPP and OSI were significantly (p<0.05) increased in BC, SP and MC when compared with the controls and followed the trend of: BC (6 folds) > SP (5.8 folds) > MC (5.2 folds) while there were no significant changes in the mean values of NO in BC, SP and MC when compared with the controls (Table 2).).

Table 1:

Blood Lead Levels and Physical Properties of Lead Exposed Workers and Controls

Study groups	Pb ²⁺ (µg/dl) (mean± SD)	Age (yrs) (mean± SD)	Exposure (yrs) (mean± SD)
BC	51.50±18.84*	46.30±10.31	23.35±8.39
SP	35.80±10.13*	35.90±5.24	17.60±7.89
MC	10.60±2.55	45.20±9.91	18.60±5.94
Controls	8.51±4.55	38.20±4.48	0
F1, p- values	43.16, <0.01*	5.26, <0.01*	3.25, <0.01*

*significantly different from control at 95% level of significance, (p< 0.05).

BC – Battery Chargers

SP – Spray Painters

MC – Auto-mobile Mechanics

The mean values of all the antioxidant enzymes were significantly (p<0.05) reduced in BC, SP and MC when compared with the controls. For example, the mean values of plasma CAT significantly decreased by 75% in all the groups and SOD activities were reduced by 73.2%, 83.5% and 33.9% in BC, SP and MC respectively. The levels of the reduced GSH were significantly decreased in BC, SP and MC in the order 27.9%, 39.7% and 45.8% when compared to the controls (Table 3). There was also positive correlation (p< 0.001), between Pb-B levels in the groups BC and SP and no correlation in the control group (r = 0.714, p = 0.000). The present study found a significant increase of erythrocyte lipid peroxide level (p<0.05) and significant decrease in the activities of erythrocyte antioxidant enzymes such as superoxide dismutase (p<0.05) and catalase (p<0.05) in the battery chargers and spray painters group as compared to the control group. A positive correlation between MDA concentration in erythrocytes and lead concentration in blood was established. Generally, on comparing the correlation relationship results of these parameters, in group 1, BC, there was a negative correlation between TAP and TPP (r = -0.19, p = 0.94), a negative correlation between TAP and OSI (r = -0.22, p = 0.38), a positive correlation between TPP and OSI (r = 0.44, p = 0.07) and a significant positive correlation (p< 0.001) between MDA (a function of lipid peroxidation) and OSI (r = 0.701, p = 0.001). In group 2, SP, there was significant positive correlation (p<0.001) between TPP and OSI (r = 0.99, p = 0.00) and a positive correlation (p< 0.001) between OSI and TPP (r = 0.971, p = 0.000).

Table 2:
Markers of Oxidative Stress in Lead Exposed Workers and Controls

Study groups	MDA (nM/ml)	TPP (mol/LH ₂ O ₂)	TAP (Trolox equiv/L)	OSI (%)	NO (μ mol/L)
BC	9.27± 0.75*	45.20±10.78*	1172.22±165.0*	14.44±1.12*	34.60±13.41
SP	8.44± 1.20*	53.39±17.52*	1205.50±142.4*	4.39±1.24*	21.50±9.07
MC	7.33± 0.47*	45.96±23.54*	1264.00±115.9*	3.82±1.83*	41.00±16.00
CONTROLS	5.11±0.83	12.67±5.18	1611.33±127.6	0.75±0.28	25.17±14.49
t _a , P _b value	9.03, 0.00*	2.08, 0.04*	4.00, 0.00*	0.83, 0.24	1.32, 0.20
t _b , P _b value	3.44, 0.02*	8.54, 0.00*	7.45, 0.00*	11.07, 0.00*	1.53, 0.10
t _c , P _c value	2.09, 0.04*	10.68, 0.00*	8.42, 0.00*	12.39, 0.00*	3.14, 0.06

*significantly different from control at 95% level of significance, (p< 0.05).

BC – Battery Chargers

SP – Spray Painters

MC – Auto-mobile Mechanics

a – BC compared with the control (a & d)

b – SP compared with the controls (b & d)

c – MC compared with the controls (c & d)

Table 3:
Enzymatic Antioxidants and Reduced Glutathione Levels (Mean ± SD) in Lead Exposed Workers and Controls

Study groups	GSH (μmol/ml)	SOD (Plasma) (Units / mg protein)	SOD (Lysate) (Units / mg protein)	CAT (plasma) (μ mole H ₂ O ₂ consumed/min/mg protein)
BC	50.89±4.39*	1.47±0.70	0.34±0.24*	0.03± 0.01*
SP	42.50±8.92*	1.42±0.14	0.21±0.17*	0.03± 0.00*
MC	38.20±8.57*	1.46±0.07	0.84±0.30*	0.03±0.00*
Controls	70.53±13.23	1.44±0.83	1.27±0.12	0.12±0.01
t _a , P _a value	5.93, 0.00*	1.01, 0.32	13.54, 0.00*	2.90, 0.01*
t _b , P _b value	3.36, 0.00*	1.19, 0.24	5.53, 0.00*	22.79, 0.00*
t _c , P _c value	5.22, 0.00*	0.25, 0.81	4.79, 0.00*	26.47, 0.00*

*significantly different from control at 95% level of significance, (p< 0.05).

BC – Battery Chargers; SP – Spray Painters; MC – Auto-mobile Mechanics

a – BC compared with the controls

b – SP compared with the controls

c – MC compared with the controls

DISCUSSION

Exposure to heavy metals is a common phenomenon due to their environmental pervasiveness. Metal intoxication particularly neurotoxicity, genotoxicity or carcinogenicity is also widely known (Flora *et al.*, 2006). The study focuses on the establishment of lead toxicity as being an underlying factor in the generation of reactive oxygen species which could lead to an imbalance between pro-oxidant and antioxidant homeostasis, usually termed as oxidative stress, in workers occupationally exposed to lead (Pb²⁺) which has gradually become a chemical terrorist that has come to stay.

The present study shows higher oxidative stress levels in lead-exposed workers. This report corroborates the previous findings that lead has direct oxidant effects on tissues, cell membranes and cellular components of animal models (Vaziri and Ding 1999b, and Puppo *et al.*, 1998). Numerous activities involving lead and its products are carried out within the city of Ibadan and in Nigeria generally. Many individuals under 20 years of age are involved in these activities as a form of earning a living. Occupational lead exposure to the underage aggravates the problem further as the young ones are prone to development of organ and systems failure at an early age. Nigerians are exposed to excessive inhalation of lead fumes, oral ingestion and dermal absorption of lead particles when dismantling disused lead accumulators, washing the lead cells

in water and smelting the cells (Abiola 2009). This study indicates that lead toxicity still persists in automobile workers and investigated the likely effects of this exposure on health Blood lead (Pb-B) levels which significantly increased in the various groups used, battery chargers (BC) and spray painters (SP) but not in automobile mechanics (AM), as compared to normal control subjects. This indicates a greater lead (Pb) absorption in these groups as compared to the control group. The biochemical basis for those toxic effects still needs to be investigated, however, activity of some membrane-bound enzymes and composition of membrane proteins in RBC were also found to be altered by lead exposure (Sharma *et al.*, 2011a). It has now become clear that high to moderate doses of lead exposure induce generation of free radicals resulting in oxidative damage to critical biomolecules such as lipids, proteins and DNA (Hande and Naran 2000). Lead causes oxidative stress by inducing the generation of ROS, reducing the antioxidant defense system of cells via depleting glutathione, interfering with some essential metal, inhibiting sulfhydryl dependent enzymes or antioxidant enzymes activities and/or increasing susceptibility of cells to oxidative attack by altering membrane integrity and fatty acid composition (Adler *et al.*, 1993). SOD, the first line of defence against oxygen derived radicals is responsible for the dismutation of superoxide radicals to H₂O₂, whereas CAT metabolically removes H₂O₂ from the intracellular environment, thereby further reducing the H₂O₂ and hydroxyl

radical generation (Isaac *et al.*, 2013). Decreased SOD and catalase activities in the BC and SP groups indicate possible lead-induced generation of O_2^- and H_2O_2 . Thus it is speculated that Pb^{2+} may induce generation of ROS by interacting with oxy-haemoglobin, which may lead to oxidative damage of RBC membrane. The decreased SOD activity in the study groups is probably related to the interaction of lead with copper, since SOD is zinc and copper containing enzyme. Lead induced copper deficiency resulting in a decrease in SOD activity and decreased scavenging of super-oxide radicals are reported (Das *et al.*, 2001). Catalase contains heme as the prosthetic group, the biosynthesis of which is inhibited by lead and resulted in decreased erythrocyte catalase activity. Heavy-metal-induced alteration of antioxidant enzyme activities and nucleic acid concentration are also reported (Das and Das 2004). GSH, a thiol group-containing molecule, is known for its effective antioxidant property by scavenging oxidative stress-inducing molecules. The main protective roles of GSH against oxidative stress include scavenging of hydroxyl radicals and singlet oxygen directly, detoxifying H_2O_2 and lipid peroxides by the catalytic action of GPx and also in regenerating antioxidant vitamin (i.e., vitamins C) back to its active form (Masella *et al.*, 2005).

The present report also agrees with Nemsadze (Menke *et al.*, 2006) who reported significantly lower levels of SOD, GSH and CAT in lead exposed workers. They associated their findings with the effects of lead toxicity on thiol-containing antioxidants and enzymes (superoxide dismutase, catalase, glutathione peroxidase, glucose 6-phosphate dehydrogenase and antioxidant molecules like GSH. Indirect depletion of GSH may occur when lead inhibits the enzyme and aminolevulinic acid dehydratase (ALAD) before it catalyzes the condensation of two molecules of d-aminolevulinic acid (δ -ALA) to porphobilinogen. When the activity of ALAD is inhibited an effect of lead exposure which has been confirmed experimentally by several authors, the amount of δ -ALA increased (Lyn Patrick 2006). Significantly higher level of oxidative stress in this study could be explained by the hypothesis that lead toxicity leads to the generation of reactive oxygen species (ROS) and a direct depletion of antioxidant. It could therefore be concluded from this study that lead plays an active role in the generation of reactive oxygen species and oxidative stress. Adjuvant antioxidant therapy may be needed to avert the consequences of oxidative stress in workers occupationally-exposed to lead. On the other hand, leaded petroleum had been identified as the largest source of lead poisoning all over the world and the Millennium Development Goals adopted at the World summit on Sustainable Development (WSSD) held in Johannesburg 2002, recommends immediate phase out of leaded petrol in all parts of the world. Apart from speeding up the complete phase out of leaded gasoline in our country, focus should also be directed towards other sources of environmental lead such as releases from industries involved in iron and steel production, lead-acid-battery and paint manufacturing, burning of solid lead-containing waste, plastics and cigarette smoke. Health care service providers should be equipped with diagnostic facilities and knowledge to appropriately attend to the problem medically. Interdisciplinary approach, bringing together the Ministries of Health, Environment, Housing, National Environment Management Authority (NEMA), children welfare organisations and all other concerned authorities is recommended.

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