BIOCHEMICAL ASPECTS OF LEAD EXPOSURE IN SILVER JEWELRY WORKERS IN WESTERN MAHARASHTRA (INDIA)

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ABSTRACT

This study was conducted to examine the effect of blood lead (Lead) on heme biosynthesis, the hematopoietic system, oxidative stress, and antioxidant status of silver jewelry workers (SJW) in western Maharastra (India). The blood lead level of the SJW group (N=30) was in the range of 30.2-64.7 μg dL^{-1} (mean±SD, 48.56 ± 7.39 μg dL^{-1}), whereas that of non-occupational Lead-exposed normal healthy control subjects (N=35) was 2.8-22.0 μg dL^{-1} (mean±SD ,12.52±4.08 μg dL^{-1}). Although the blood lead level of the SJW group increased significantly ($p<0.001$) when compared with the control group, the urinary excretion of lead in the SJW group was not altered. In the SJW group, non-activated δ-aminolevulinic acid dehydratase (ALAD) activity significantly decreased ($p<0.05$), and the ratio of activated/non-activated ALAD increased when compared with controls ($p<0.001$), whereas activated ALAD activity was not altered significantly. Erythrocyte zinc protoporphyrin (ZPP) was not altered in SJW. The urinary excretion of δ-amino-levulinic acid ($p<0.001$) and porpobilinogen ($p<0.05$) of the SJW group increased significantly when compared with controls. Hematology parameters, such as the packed cell volume ($p < .001$) and total erythrocyte count ($p<0.05$) significantly decreased, whereas the mean corpuscular hemoglobin concen-

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tration ($p < .001$) and total white blood cell count ($p < .001$) increased in the SJW group in comparison with controls. The serum malondialdehyde content significantly increased ($p < .001$), and the activities of antioxidant enzymes erythrocyte-SOD ($p < .001$), erythrocyte catalase ($p < .05$), and plasma ceruloplasmin ($p < .001$) significantly decreased in the SJW group compared with the controls. The results of the study clearly show an alteration of heme biosynthesis and cellular impairment of the pro-oxidants/antioxidants balance, resulting in oxidative damage in the silver jewelry workers group in western Maharashtra (India).

KEYWORDS

Lead-B, Lead-U, ALAD, ZPP, ALA-U, LEADG-U, lipid peroxidation, SOD, catalase, ceruloplasmin

INTRODUCTION

Lead, a ubiquitous and versatile metal that has been used by humankind for over 6000 years, is today one of the most widely distributed toxins in the environment. Lead and its compounds can enter into the environment at any point during mining, smelting, processing, use, recycling, or disposal. The metal is mainly used in acid batteries, cable sheathing, in color pigments, petrol additives, soldering water distribution pipes, ceramic glazes, paper industries, printing press, and silver jewelry industries /1–2/. Lead that is present in food, beverages, soil/dust, and atmospheric air is absorbed by the gastrointestinal tract (GIT). The metal is rapidly taken up in blood and soft tissues (half-life 28-36 days) then to bone (half-life 27 years). Lead has been shown to cause adverse effects in several organs and organ systems, including the hematopoietic, nervous, renal, cardiovascular, reproductive, and immune and is mutagenic in mice /1–3/. The biological effects of lead are depend upon the level and duration of exposure. Lead inhibits three enzymes of heme biosynthesis—δ-amino-levulinic acid dehydratase (ALAD), coproporphyrin oxidase, and ferrochelatase /2/. The metal affects erythrocyte formation by impairing globulin and heme synthesis and depresses serum levels of erythropoietin, a hormone that regulates erythrocyte

formation. Lead also decreases erythrocyte survival through its inhibition of membrane-bound Na^+/K^+-ATPase, resulting in decreased hemoglobin synthesis and anemia in children and adults /1,4/. Several studies have reported that metals, especially iron, copper, nickel, and lead produce reactive oxygen species (ROS), leading to lipid peroxidation, DNA damage, and depletion of the cell's antioxidant defense system /5/. Erythrocytes are more vulnerable to oxidative damage than are many other cells /6–8/. Over 95% of blood lead is bound to erythrocytes and seems to be in dynamic equilibrium with plasma lead /9/. Lead causes an alteration in antioxidant enzyme activities, such as superoxide dismutase (SOD), catalase, glutathione peroxide, and changes in the concentration of certain antioxidant molecules like glutathione (GSH) in lead-exposed animals /10–11/and workers /12–15/. Indian silver jewelry makers—who engage in silver refining from waste by lead smelting, alloying, rolling, and milling silver wires and pieces; designing, polishing, and plating silver jewelry—have been found to have high blood lead levels compared with unexposed controls /16/. As most research on lead exposure and erythrocyte antioxidant enzymes activity has been experimental, and the results often divergent, the purpose of this study was to evaluate the activity of these enzymes and the concentration of products of ROS in blood with reference to heme biosynthesis in silver jewelry workers (SJW) of Western Maharashtra (India) exposed to lead compounds.

EXPERIMENTAL

The study group included 35 non-occupationally lead-exposed healthy male subjects from rural areas and 30 occupational lead-exposed silver jewelry (rings) making workers from Kolhapur (Western Maharashtra). All subjects were in the range of 20-40 years of age. The demographic, occupational, and clinical data were collected from the study and control subjects by a questionnaire and interview before blood and urine sample collection. The average socioeconomic status, normal dietary intake, and food habits, nonsmokers, nonalcoholic healthy male subjects of the study and control groups were taken for this study. The subjects who were on drugs for minor illness and past history for minor illness were excluded from this study. Silver ring making and refining units workers who were

exposed to lead for more than 6 h per day, with a duration of lead exposure from 2 to 20 years, were selected for this study. The entire experimental protocol was approved by institutional ethical committee, and utmost care was taken during the experimental procedure according to the Helsinki declaration of 1964 /17/. Blood samples were collected by puncturing the anticubital vein into evacuated tubes containing heparin solution as anti-coagulant. At the time of blood collection, random urine samples were collected in small black-colored plastic bottle to avoid errors from the inadequate collection of 24 h in urine samples from each subjects. The blood lead and urinary lead were measured using a Perkin Elmer model 303 graphite furnace atomic absorption spectrophotometer, which was connected to Hitachi 165 recorder; values were shown in $\mu g\ dL^{-1}$ /18/. Erythrocyte δ-aminolevulinic acid dehydratase (ALAD) was estimated by the Julian Chisolan method /19 /. Erythrocyte ALAD acts on aminolevulinic acid (ALA) to form porphobilinogen (PGB), which is further reacted with modified Ehrlich's reagent to form a pink-colored compound measured on a spectrophotometer at 555 nm. Hg-TCA solution stops the reaction by precipitating the proteins. ALAD activity is estimated by using this formula.

$$\text{ALAD activity (μMol ALA utilized/min/L of erythrocytes)} = \frac{\text{Net absorbance} \times 100 \times 2 \times 35}{\text{(\% Hematocrit x 60x 0.062)}}$$

where

2	=	Conversion factors for ALA to PBG
35	=	Dilution factor
60	=	Incubation time (min)
0.062	=	Micro-molar absorptivity of modified Ehrlich's Reagent and PBG chromogen

Erythrocyte ALAD activated by zinc acetate and ratio of activated/non-activated ALAD (Act/Non-act) was determined. The erythrocyte ZPP concentration was measured directly by using an Aviv biomedical hematoflurometer model 206 /20/. δ-Aminolevulinic acid (ALA) was estimated in urine samples by the method of Osamu et al /21/. δ-Aminolevulinic acid reacts with acetyl acetone and forms pyrrole substance, which reacts with p-dimethyl amino benzaldehyde. The colored complex was measured spectrophotometrically at 555 nm. The results were

expressed as mg L^{-1}. Porphobilinogen in urine was estimated according to Mauzerall and Granick /22/. Porphobilinogen from urine reacts with p-dimethyl aminobenzaldehyde (DMAB, Ehrlich's reagent) in acid solution to form a red compound, which is measured at 555 nm after exactly 5 min; the values were calculated according to the Rimington formula /23/.

$$\text{Urinary PBG } (\text{mg } L^{-1}) = \frac{\text{OD x Numbers of times the urine diluted}}{70.85}$$

All hematological parameters were measured using a fully automated Hematology analyzer Sysmax K-4500 /24/. Lipid peroxidation was measured spectrophotometrically by method of Kei Satoh /25 /. Serum proteins were precipitated by trichloroacetic acid (TCA) and the mixture is heated for 30 min with thioburbituric acid in 2M sodium sulfate, in a boiling water bath. The resulting chromogen is extracted with n-butyl alcohol and the absorbance of the organic phase is determined at a wavelength of 530 nm. The values are expressed in terms of malondialdehyde (MDA) nmol mL^{-1} using 1, 1, 3, 3, tetraethoxypropane as the standard. The activity of erythrocyte superoxide dismutase (SOD) was measured by the method of Marklund and Marklund /26 /. Superoxide anion is involved in the auto-oxidation of pyrogallol at alkaline pH 8.5 and is inhibited by SOD, which can be determined as an increase in absorbance per two minutes at 420 mm. The SOD activity was measured as units mL^{-1} hemolysate. One unit of SOD is defined as the amount of enzyme required to cause 50% inhibition of pyrogllol auto-oxidation. Erythrocyte catalase was measured by the method of Aebi /27/. Heparinized blood was centrifuged and plasma was removed, and the erythrocytes were washed 2-3 times with 0.9% NaCl and then lysed in 10 volumes of cold deionized water. The whole mixture was centrifuged for 10 min at 3000 rpm. The cell debris was removed and the clear hemolysate was diluted 500 times with phosphate buffer (60 mM) pH 7.4. Catalase decomposes H_2O_2 to form water and molecular oxygen. In the UV range, H_2O_2 show a continual increase in the absorption with decreasing wavelength. At 240 nm, H_2O_2 absorbs maximum light. When H_2O_2 is decomposed by catalase, then the absorbance decreases. The decreased absorbance was measured at 240 nm for every 15 seconds interval up to 1 min and the difference in absorbance (ΔA at 240 nm) per unit time is a measure of the catalase activity. The unit of catalase activity was expressed

as mM of H_2O_2 decomposed mg Hb min^{-1}. Plasma ceruloplasmin was measured by method of Ravin /28/. Ceruloplasmin oxidizes P-phenyl-enediamine in presence of oxygen to form a purple-colored oxidized product. The cerulplasmin concentration was determined from the rate of oxidation of P-phenylenediamine at 37°C at pH 6.0, which has an absorption peak at 530 nm. Statistical analyses between the control and SJW groups were done using the unpaired student's *t* test.

RESULTS

Table 1 summarizes the lead concentration in blood (Pb-B), in urine (Pb-U), activated and nonactivated erythrocyte ALAD and its ratio, erythrocyte ZPP, ALA in urine (ALA-U), and porphobilinogen in urine (PBG-U) in the lead-exposed SJW group and in the normal healthy control group.

TABLE 1

Pb-B, Pb-U and parameters related to heme biosynthesis in study and control groups

Parameters	Group	
	Unexposed controls (N=35)	Silver jewellery workers (N=30)
Pb-B (μg dL^{1})	12.52±4.08 (2.8-22.0)	48.56±7.39 (30.2-64.7)**
Pb-U (μg dL^{1})	6.97±3.59 (1.0-13.2)	9.39±6.50 (2.0-30.7)#
ZPP (μg dL^{1})	8.25±7.79 (1.0-35)	9.55±3.21 (1.0-11)#
δ-ALA-U (mgL^{-1})	9.62±5.45 (2.5-17.5)	25.13±9.52 (9.37-50)**
PBG-U (mgL^{-1})	10.10±2.87 (3.5-15.87)	12.06±4.81 (7.05-24.7)*
Ery-δ-ALAD (μmol ALA utilized/min/L RBC)		
Act	27.48±14.94 (9.59-87.72)	25.78±6.35 (13.70-41.40)#
Non act	24.2±13.50 (8.34-78.95)	19.23±5.30 (10.04-32.20)*
Act/ Non act	1.14±0.081 (1.02-1.38)	1.37±0.298 (1.0-2.33)**

** P<0.001, * P<0.05, # non significant as compared with control group. Pb-B, Blood lead concentration; Pb-U Urinary lead concentration, ALAD, δ-Aminolevulinic acid dehydratase; ZPP, Zinc protoporphyrin; ALA-U, urinary δ-aminolevulinic acid; PBG-U, Urinary porphobilinogen.

The Pb-B level of normal healthy control group ranged from 2.8-22.0 μg dL^{-1}, and for the lead-exposed SJW group 30.2-64.7 μg dL^{-1}. The Pb-B ($p < 0.001$) was significantly increased in the SJW group as compared with the unexposed control group, whereas Pb-U was not significantly altered in the SJW. Non activated erythrocyte ALAD activity was significantly decreased ($P < .05$) in the SJW group as compared with the control group. However, the activated erythrocyte ALAD activities in SJW group showed no significant change when compared with the control group. The ratio of activated/non activated erythrocyte ALAD activities showed a significant increase ($p < .001$) in the SJW group as compared with the control group. The erythrocyte ZPP level was not significantly altered in SJW group. Both ALA-U and PBG-U of the SJW group showed a significant elevation ($p < .001$ and $p < .05$) above their respective control groups. **Figure 1** shows the percent change in Pb-B, Pb-U, activated, nonactivated and ratio of activated/non activated erythrocyte ALAD, ALA-U, and PBG-U of the lead-exposed SJW group compared with unexposed control groups. **Table 2** shows the hematological parameters of the lead-exposed SJW and unexposed control groups.

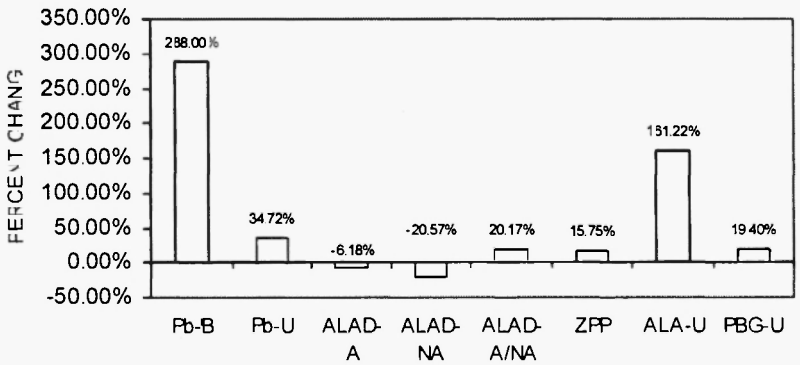

Fig. 1: Percent change chart of lead concentration in blood (Pb-B),lead concentration in urine (Pb-U), activated erythrocyte, δ-aminolevulinic acid dehydratase (ALAD-Act) non-activated erythrocyte δ-aminolevulinic acid dehydratase (ALAD-NA), activated and non-activated erythrocyte δ-Amino levulinic acid dehydratase ratio (ALAD-A/NA), erythrocyte-zinc Protoporphyrin (ZPP),δ-aminolevulinic acid in urine (ALA-U) and porphobilinogen in urine (PBG-U) of lead exposed silver jewelry worker group of Western Maharashtra (India) with respect to control group.

TABLE 2

Hematological parameters of the silver jewellery workers and control groups.

Parameters	Group	
	Unexposed controls (N=35)	Silver jewellery workers (N=30)
Hb (g L^{-1})	14.36±0.92 (12.6-16.3)	13.82±1.14 (11.7-15.5)
PCV (%)	45.19±2.29 (40.4-50)	42.69±2.73 (35.7±50.3)**
MCV (fl)	88.77±5.05 (76.7-109.7)	87.76±3.85 (77.4-99.5)
MCH (pg)	29.73±2.05 (26.1-36.6)	29.60±1.18 (22.6±35.2)
MCHC (g dL^{-1})	31.89±0.89 (29.3-33.3)	32.89±0.99 (29.2-35.4)**
Total erythrocytes (million μL^{-1})	5.10±0.43 (4.26-5.71)	4.83±0.42 (3.73-5.58)*
Total leucocytes (mm^3)	6.34±1.66 (4.2-8.9)	7.86±1.42 (6.0-11.2)**

** P<0.001, * P<0.05. Hb, hemoglobin concentration; PCV, Packed cell volume; MCV, mean corpuscular volume; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration

TABLE 3

Lipid peroxide (MDA) superoxide dismutase (SOD), catalase, ceruloplasmin in silver jewelry workers and control group

Parameters	Group	
	Unexposed controls (N=35)	Silver jewellery workers (N=30)
Malondialdehyde (nmol mL^{-1})	1.12±0.480 (0.55-2.22)	1.496±0.29 (1.11-2.4)**
SOD (unit mL^{-1} hemolysate)	14.12±3.39 (4.57-19.42)	10.51±2.53 (6.22-14.22)**
Catalase (mM H_2O_2 decom mg Hb min^{-1})	47.63±17.94 (16.90-97.18)	39.77±10,92 (16.90-59.15)**
Ceruloplasmin (mg dL^{-1})	53.17 ± 16.03 (11.37-83.12)	38.67±8.51 (21-56.87)**

**P<0.001, * P<0.05

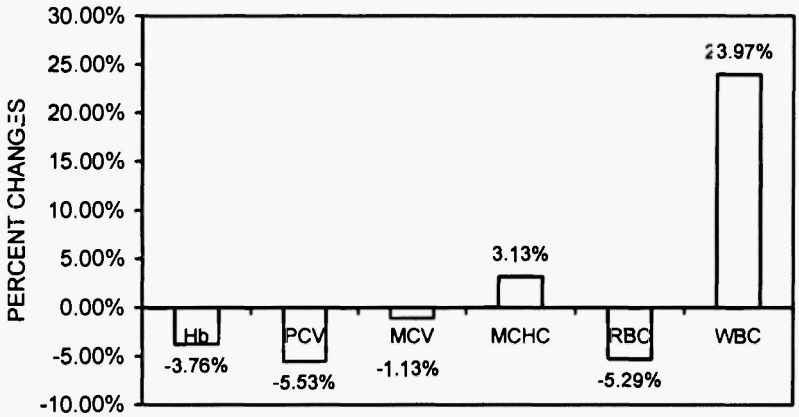

Fig. 2: Percent change chart of hemoglobin (Hb) concentration, packed cell volume (PCV), mean corpuscular volume(MCV), mean corpuscular hemoglobin concentration(MCHC), red blood corpuscle (RBC), and white blood corpuscle (WBC) of lead exposed silver jewelry workers (SJW) group of western Maharashtra in comparison to their respective controls.

A significant decrease of PCV ($p < .001$), total erythrocytes counts ($p < .05$), increase of mean corpuscular hemoglobin concentration ($p < .001$), total leukocyte count ($p < .001$), and unaltered values of Hb, mean corpuscular volume (MCV) and mean corpuscular hemoglobin (MCH) were observed in the SJW group as compared with their respective controls. The percent change chart of various hematological parameters IS shown in Fig. 2. Table 3 depicts the serum MDA content, erythrocyte SOD, erythrocyte catalase activities and plasma ceruloplasmin in lead exposed SJW group. The lipid peroxidation level (MDA) was significantly increased ($p < 0.001$) and the antioxidant enzymes activities such as erythrocyte SOD ($p < .001$), erythrocyte catalase ($p < 0.02$) and plasma ceruloplasmin ($p < 0.001$)were significantly decreased in SJW group as compared to control group.

Figure 3 shows the percent change difference of serum lipid peroxide (MDA), erythrocyte SOD, erythrocyte catalase and plasma ceruloplasmin activities in SJW group as compared to control group.

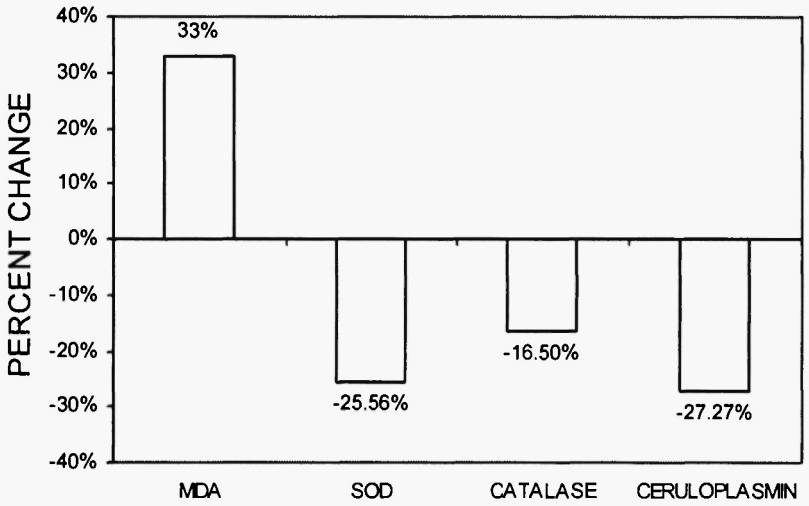

Fig. 3: Percent change chart of serum malondialdehyde (MDA), erythrocyte-superoxide dismutase (SOD) and erythrocyte- catalase (CAT), plasma ceruloplasmin activities of lead exposed Silver Jewelry Workers (SJW) group of Western Maharashtra (India) with respect to control group.

DISCUSSION

The significant elevation of blood lead (Pb-B) level but unaltered urinary lead (Pb-U) level in silver jewelry workers as compared with normal control subjects could be due to a greater excretion of lead though sweating. The silver refining unit workers were exposed to high temperature due to inadequate ventilation and greater room temperature at their working places. Normally lead absorption results in rapid urinary excretion. If excessive lead exposure continues, the metal accumulates in bone. If bone storage capacities are exceeded, then lead shifts into soft tissues. The Pb-B levels depend on the equilibrium between absorption, storage, and excretion /1-2/. The Pb-B level generally reflects acute (current) exposure because of the short half-life for lead in blood (28 to 36 days), but it is also influenced by previous storage. The Pb-B level is the best and most sensitive biomarker for identifying lead pollution, human exposure, and its adverse effects. Yet, single Pb-B measurement does not show the body burden of lead as the

metal is circulating in three compartments—blood, bone, and soft tissues /1/. The study indicates a greater absorption of lead in SJW group (range 30.2 to 64.7 μg dL^{-1}) as compared with the control group (range 2.8 to 22.0 μg dL^{-1}).

The results from the alteration of non-activated erythrocyte ALAD and activated/non activated ALAD activities clearly indicate the inhibition of heme biosynthesis. In several studies reported, lead induced the inhibition of δ-ALA dehydratase, coproporphyrin oxidase, ferrochelatase activities, and increased δ-ALA synthase activity leads to increased production and excretion of the PBG, δ-ALA, and coproporphyrin in association with increased circulatory protoporphyrin level /2/. In this study, non-activated erythrocyte ALAD decreased by 20.57%, and the ratio of activated/non-activated ALAD increased by 20.17% in SJW as compared with the controls. We also observed an increased excretion of ALA and PBG in urine by 161.22% and 19.40%, respectively, in SJW as compared with controls. The results suggest that lead inhibits the δ-ALAD dehydratase enzyme of heme biosynthesis. The lead-induced decreased activities of ALAD in the SJW group could be due to its interaction with enzyme functional groups or high-affinity metal-binding proteins, such as lead-binding proteins and metallothioneins or both /29/. The level of ALAD is a sensitive indicator of lead toxicity and is usually reduced to 50% or less of normal activity when Pb-B values are in the 30 to 50 μg dL^{-1} range. Unfortunately, the erythrocyte ALAD activity reached a plateau when marked reductions of it took place; hence it cannot be used to quantify the degree of lead exposure /2/. Non-activated erythrocyte ALAD alone is considered as a predictor of Pb-B concentration as per the European standardized and other similar ALAD assay methods /28/. Several studies have reported that the erythrocyte ALAD activity is increased in individuals with anemia and sickle cell disease but not in subjects with β-thalassemia /30/. Therefore, the use of the activated/non activated erythrocyte of δ-aminolevulinic acid dehydratase (ALAD) activity ratio appears to be good marker for lead toxicity. Lead induced inhibition of the Zn-containing enzyme ALAD activity resulted in decreasing catalyzing the condensation of two units of ALA to form PBG and increase the δ-ALA in plasma and urinary excretion. The ALA is synthesized in mitochondria from glycine and succinyl CoA by ALA synthetase (ALA-S), a rate limiting enzyme in the heme biosynthesis pathway. The decrease in ALAD activity and indirect activation of ALAS due to negative feedback regulation by lead exposure causes an increase in

ALA in various tissues and in plasma and consequently an increase in the excretion of ALA in urine /31/. Hence, the measurement of ALA-U is useful for biological monitoring of occupational lead exposure. Hematological parameters such as PCV ($P < .001$), erythrocyte count ($P < .05$) were decreased and in MCHC ($P < .001$), total leukocyte count ($P < .001$) were increased, whereas Hb, MCV, MCH were not altered in SJW as compared with controls. The effects of lead on the hematopoietic system resulting in a decrease of Hb synthesis and anemia was observed in children and adults /2/. Lead affects the hematopoietic system at several levels, i.e. inhibiting heme and globin synthesis, erythrocyte formation, decreased serum levels of erythropoietin, a hormone that regulate erythrocyte formation, and also decreases erythrocyte survival through its inhibition of membrane-bound Na^+- K^+-ATPase /1, 2, 32/. Several studies have shown that poisoning due to heavy metals such as lead produces high leukocyte counts /33/. Several experimental and clinical studies reported that lead increased the concentration of lipid peroxide in blood /34–35/. It was also reported that increased levels of lipid peroxide in plasma is associated with decreased erythrocyte SOD and catalase activities in occupationally lead-exposed workers /36/. Lead can causes oxidative stress through various mechanisms, i.e. acceleration prooxidants formation and reducing the antioxidant defense system of cells via depleting glutathione, interfering with some essential metals, inhibiting sulfhydryl-dependent enzymes or antioxidant enzymes activities and/or increasing the susceptibility of cells to oxidative attack by altering membrane integrity and fatty acid composition /6/. Lead inducing the generation of reactive oxygen species (ROS) was reported in experimental in vitro and in vivo studies /37/. The results of past studies and the present study indicate the possible involvement of ROS in lead-induced toxicity. However, the mechanisms by which lead induces oxidative stress are poorly understood. The evidence indicates an involvement of multiple mechanisms. As 95% lead is present in erythrocytes, the metal alters RBC membrane structure and functions /38/. Several studies reported that erythrocytes are more vulnerable than any other cells to oxidative damage /7-9/. The interaction of heavy metals with oxyhemoglobin has already been suggested as important source of superoxide formation in erythrocytes /39/. The decreased erythrocyte SOD ($P < .001$, 25.56%) and catalase ($P < .02$, 16.50%) activities seen here in the SJW group indicate increase the generation of ROS by interacting with the oxygen in hemoglobin, leading to

peroxidative damage of the cell membrane The increased white blood cells count ($P < .001$) in SJW as compared with controls can also be considered for the rise in serum MDA in SJW group because free radicals released from activated leukocytes can induce the peroxidation of membrane lipids. Hence increase serum MDA could be used as a marker for the free-radical-mediated destruction of liver parenchymal cells /40/. Lead may impair the antioxidant status of the SJW group, which leads to oxidative stress. The decreased SOD activity in the SJW group is probably related to the interaction of lead with copper as SOD is a zinc and copper containing enzyme. Lead-induced copper deficiency and decreased SOD activity, resulting in decreased scavenging of superoxide radicals, has been reported in several studies /41–43/. Catalase contains heme as the prosthetic group, the biosynthesis of which is inhibited by lead, resulting in decreased erythrocyte catalase activation. Heavy-metal-induced alteration of anti-oxidant enzyme activities and nucleic acid concentration are also reported /44–45/. Hence, whether these alterations are the cause of the oxidative damage or it's effects on the genetic materials which are responsible for the synthesis of endogenous antioxidant enzymes system of cells is not clear. Possibly lead may initially affect some components of the cellular anti-oxidant defense system, which might cause impairment in the pro-oxidant /antioxidant balance in cells resulting in oxidative damage /9/. Ceruloplasmin is a copper-containing α_2 glycoprotein with enzymatic activity as ferroxidase. Ceruloplasmin is a multifunctional enzyme and performs many physiological functions like copper storage, ascorbate oxidase activity, oxidation of nitric oxide, as well as pro-oxidant and antioxidant activity. Ceruloplasmin also plays a crucial role in iron metabolism, whereby it assists the release of iron from cells before its uptake by transferrin /46/. The enzyme converts Fe^{2+} to Fe^{3+} and removes Fe^{2+} from the blood, which could otherwise become involved in the generation of harmful ROS.

In conclusion, it may be said that the increased Pb-B level in SJW affects heme biosynthesis, the hematological system, and impairs the pro-oxidants/antioxidants balance of cells resulting in oxidative damage. Therefore, additional supplementation of antioxidant vitamins like ascorbic acid, α-tocopherol, β-carotene, and GSH could be beneficial to lead-exposed workers /47 /.This study also reveals that a complete hemogram, urinary

ALA, PBG, Pb-B, Pb-U, and erythrocyte δ-ALAD activities are valuable tools in screening for occupational lead exposure.

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