Biochemical aspects of lead exposure and toxicity in spray painters of Western Maharashtra (India)

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Abstract

The present study was undertaken to assess biochemical, hematological and antioxidant status of possible lead exposed spray painters of Western Maharashtra (India). Thirty spray painters (SP) and thirty five normal healthy subjects were taken (age 20 - 40 years) from the Western Maharashtra for this study. Venous blood samples and random urine samples were collected from both the groups. The blood Pb level of SP group (N = 30) was found to be in the range of 7.5 to 45.7 microgram/dL (Mean ± SD, 22.32 ± 8.87 microgram/dL) whereas that of the unexposed control group (n= 35) was in the range of 2.8 - 22.0 microgram/dL (Mean ± SD, 12.52 ± 4.08microgram/dL). The blood lead level (Pb-B) and urinary lead level (Pb-U) were significantly increased in SP group as compared to control group. Though activated and non-activated erythrocyte-ALAD activities in SP group did not show any significant change as compared to the control group, the ratio of activated/non-activated erythrocyte ALAD activities in SP group showed a significant increase ($p<0.001$). Erythrocyte zinc protoporphyrin (ZPP) level was not altered in SP group as compared to control group. A significant elevation of urinary d-aminolevulinic acid (ALA-U) and porphobilinogen (PBG-U) were observed in the SP group. A positive correlation ($r = 0.45$, $p< 0.001$) between Pb-B and ALA-U was found in the SP group, but no significant correlation was observed in the control group. Hematological parameters were not altered significantly in the SP group as compared to control group except a significant increase ($p<0.05$) in mean corpuscular hemoglobin concentration (MCHC). The serum malondialdehyde (MDA) content was significantly increased ($p<0.001$) and the activities of antioxidant enzymes such as erythrocyte superoxide dismutase (SOD) ($p< 0.001$) and erythrocyte catalase ($p< 0.05$) were significantly reduced in the SP group as compared to control group.

Therefore the study clearly indicates an adverse effect of lead on heme biosynthesis and imbalance of pro-oxidant/antioxidant status in spray painters from Western Maharashtra (India) associated with increase lipid peroxidation in association with decreased erythrocyte SOD and catalase activities.

Key words:
Environmental health; lipid peroxidation; spray painters; Pb-B; Pb-U; ALAD; ZPP; ALA-U; PBG-U.

Introduction

Lead (Pb) is one of the most widely scattered toxic metals in the world. It is believed that lead has been used by mankind for over 9000 years. Lead in the environment may be derived from natural or anthropogenic sources. Lead and its compounds may enter the environment at any point during mining, smelting, processing, use, recycling or disposal. Airborne lead can be deposited on soil and water thus reaching humans through the food chain and drinking water. Levels of lead found in air, food, water, soil and dust vary widely throughout the world and depend upon the degree of industrial development, urbanization and lifestyle factors (WHO, 1995; ATSDR, 2005). Lead is absorbed by the GIT via food, beverages, soil and dust. Dietary factors, nutritional status, chemical form of the metal and patterns of food intake affect lead absorption. Lead is not distributed homogeneously throughout the body. It is rapidly taken up in blood and soft tissues (half life 28-30 days) followed by a slower redistribution to bone (half life 27 years). Dietary and airborne lead which is not absorbed in GIT is excreted in faeces.

In humans, lead can cause a wide range of biological effects depending upon the level and duration of exposure. It causes adverse effects in several organs and organ systems including nervous, renal, cardiovascular, reproductive, hematological and immune system (ATSDR, 2005; Patil et al, 2006). Lead interferes with heme biosynthesis by altering the activity of three enzymes - -aminolevulinic acid synthetase (ALAS), - amino levulinic acid dehydratase (ALAD) and ferrochelatase. Lead indirectly stimulates the mitochondrial enzyme ALAS, which catalyzes the condensation of glycine and succinyl Co-A to form - amino levulinic acid or ALA (WHO, 1995; ATSDR, 2005). Lead also inhibits the zinc containing cytosolic enzyme ALAD, which catalyzes the condensation of two units of ALA to form porphobilinogen. This inhibition is noncompetitive and occurs through the binding of lead to vicinal sulfhydryls at the active sites of ALAD. Lead bridges the vicinal sylfhydryls, whereas Zn, which is normally found at the active sites, binds to only one of these sulfhydryls (WHO, 1995; ATSDR, 2005). The activity of ALAD, an enzyme occurring early in the heme synthesis pathway, is negatively correlated with Pb-B between 5 to 95 microgram/dL. Although inhibition of ALAD occurs at very low exposure level it may or may not affect hemoglobin concentration. The end results of these changes in enzyme activities are increased urinary porphyrins, coproporphyrin, d-aminolevulinic acid (ALA)

and increased erythrocyte protorporphyrin (WHO, 1995; ATSDR, 2005, Patil et al 2006). Lead has been known to alter the hematological system. The anemia induced by lead is microcytic and hypochromic and results primarily from both inhibition of heme and globin synthesis and shortening of the erythrocyte lifespan. Lead depresses serum level of erythropoietin; a hormone that regulates erythrocyte formation which might also affect the reserve capacity for erythrpoiesis (Dresner, 1982; WHO, 1995). Lead causes oxidative stress by inducing the generation of reactive oxygen species (ROS), reducing the antioxidant defence system of cells via depleting glutathione, interfering with some essential metals, inhibiting sulfhydryl, dependent enzymes or antioxidant enzymes activities andor increasing susceptibility of cells to oxidative attack by altering membrane integrity and fatty acid composition (Hande and Nuran, 2005).

In many countries of the world, occupational exposure to lead which results in poisoning both moderate and clinical symptomatic, is still common. Occupational exposure is entirely unregulated in many developing countries and little monitoring is conducted in developed countries (Verrula and Noah, 1990). In 18th, 19th & 20th centuries, the worst outbreaks of lead poisoning were occupational in origin. The workers who are exposed to lead absorbed from inhalation of fine lead dust or fumes, contamination of food eaten at the workplace or by absorption through the skin (Buchet et al, 1991). Lead is used in various paints because of its anticorrosive properties. In India, 10% of total lead metal utilised is used in the manufacture of paints. Wherever such paints are used, there will be potential hazards for human lead exposure. Spray painters and children are at high risk of lead exposure because of maximum uses of painted objects in residential setting in India (St. John's Medical College, 1999). Although the Government of India has officially banned the use of lead in paints, lead-containing paints are still available in the market. Hence the routine evaluation of lead toxicity (if any) among spray painters is genuinely needed.

The current study aimed to assess lead toxicity by evaluating various biochemical, hematological parameters and antioxidant status in spray painters from Western Maharashtra (India).

Method

The study group included non-lead exposed healthy male subjects and occupational lead exposed spray painters of Kolhapur city in the Western Maharashtra state of India. The occupational lead exposed group consisted of 30 male spray painters, with an age range of 20 to 40 years. Thirty-five normal healthy non occupational-lead-exposed male control subjects of the same age group were taken from the rural area. Prior to biological specimen collection, demographic, occupational and clinical data were collected from the study and control subjects by using questionnaire and interview. The socio economic status of all the subjects of study and control groups was average. None of the subjects had a past history of major illness. Dietary intake and food habits of all subjects were normal. The subjects who were on drugs for minor illnesses were excluded from this study. Non-smokers, non-alcoholic healthy male spray painters (SP), who are occupationally exposed to lead for more than six hours per day with duration of exposure of 10 to 20 years, were alone selected for this study. The entire experimental protocol was approved by an institutional ethical committee and utmost care was taken during the experimental procedure according to the Declaration of Helsinki, 1964 (1996). Blood samples were collected by anticubital vein puncture using 5 ml sterilised disposable syringe into 10 ml evacuated tubes containing heparin solution as anticoagulant to obtain erythrocytes. Random urine samples were also collected to avoid the errors from the inadequate collection of 24 hours urine samples from each subject. Analyses of lead in blood and urine were carried out by graphite furnace atomic absorption spectrophotometer (AAS) using Perkin-Elmer model 303 fitted with a boiling three-slot burner. The AAS was connected to Hitachi 165 recorder and values were expressed in microgram/dL (Parson and Slavin, 1993). Erythrocyte aminolevulinic acid dehydratase (ALAD) activities were estimated by the method of Chisolm *et al.,* (1986). Erythrocyte ALAD acts on d-aminolevulinic acid (ALA) to form porphobilinogen, which reacted with modified Ehrlich's reagent to form pink coloured compound which is measured on spectrophotometer at 555 nm. Hg-TCA solution is used to stop the reaction by precipitating the proteins. ALAD activity is calculated by using the following formula:

$$\text{ALAD (m mol ALA utlised /min/L of erythrocytes)} = \frac{\text{Net Absorbance} \times 100 \times 2 \times 35}{\%\ \text{hematocrit} \times 60 \times 0.062}$$

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 was determined. Erythrocyte ZPP concentration was measured directly using Aviv biomedical hematoflurometer model 206 (Wayne, 1996). d-aminolevulinic acid (ALA) was estimated in urine samples by the method of Osamu *et al.,* (1969). ALA reacts with acetyl acetone at 1000c at pH 4.6 and forms pyrrole, which reacts with Ehrlich reagent to form a red colour. This red colour complex was extracted with chloroform, leaving other Ehrlich positive substances in the water phase, which was measured spectrophotometrically at 555 nm. The results were expressed as microgram/L. The porphobilonogen (PBG) in urine was estimated by Mauzerall and Granick (1956). PBG in urine reacts with P-dimethyl aminobenzaldehyde in acid solution to form a red compound which was measured at 555 nm exactly after five minutes and the values were calculated according to Rimington formula (1958).

$$\text{Urinary PBG (microgram/L)} = \frac{\text{O.D x number of times urine diluted}}{70.85}$$

All the hematological parameters were measured by using fully automated hematology analyser Sysmax K-4500 (Trester,1999). Lipid peroxidation was measured spectrophotometrically by the method of Kei and Satoh (1978). The proteins in serum are precipitated by trichloracetic acid (TCA) and the mixture is heated with thiobarbituric acid (TBA) in 2 M sodium sulfate, in a boiling water bath for 30 minutes. The resulting chromogen was extracted with n-butylalcohol and absorbance of organic phase was determined at 530 nm wavelength. The values are expressed in terms of malondiadehyde (MDA) nmol/ml by using 1, 1, 3, 3, tetraethoxy propane as the standard. The activity of erythrocyte superoxide dismutase (SOD) was measured by the Marklund and Marklund method (1984). Super oxide anion is involved in auto-oxidation of Pyrogallol at alkaline pH 8.5.SOD inhibits the auto-oxidation of pyrogallol, which can be determined as an increase in absorbance per two minutes at 420 nm on a spectrophotometer. SOD activity was expressed as unit/ml of hemolysate. One unit of SOD is defined as the amount of enzymes required to cause 50% inhibition of pyrogallol auto-oxidation. Erythrocyte catalase was measured by the method of Aebi (1984). Heparinized blood was centrifuged to separate plasma and RBCs were washed two to three times by 0.9% NaCl and lysed in 10 volumes of cold de-ionized water and then 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 the H2O2 and forms water and molecular oxygen. H2O2 absorbs maximum light at 240 nm. When H2O2 is decomposed by catalase, the absorbance decreases. The decrease in absorbance was measured at 240 nm at an interval of 15 seconds for one minute. The difference in absorbance (DA at 240 nm) per unit time is a measure of the catalase activity. The catalase activity was expressed as mM of H2O2 decomposition/microgram Hb/min. Plasma

Table 1.0: Pb-B, Pb-U and parameters related to heme bio-synthesis in spray painters and control group

Groups	PbB µg/dL	PbU µg/dL	Ery-δ-ALAD (mol–ALA utilized/min /L of ery)			ZPP µg/dL	-ALA-U mg/L	PBG-U mg/L
			Act	Non-Act	Act/Non-Act			
Control groups (N-35)	12.52 ± 4.08 (2.8 - 22.0)	6.97 ± 3.59 (1.0-13.2)	27.48 ± 14.94 (9.59 - 87.72)	24.21 ± 13.50 (8.34 - 78.95)	1.14 ± 0.081 (1.02 - 1.38)	8.25 ± 7.79 (1.0 - 35)	9.62 ± 5.45 (2.5 - 17.5)	10.10 ± 2.87 (3.5 - 15.87)
Spray painters (N-30)	22.32 ± 8.87*** (7.5 - 45.7)	11.22 ± 7.15** (1.5 - 30.2)	26.67 ± 7.40 # (12.22 - 41.63)	20.60 ± 7.49 # (6.98 - 36.46)	1.34 ± 0.197*** (1.069 - 1.75)	10.45 ± 3.33 # (1.0 - 57)	15.67 ± 4.24*** (8.12 - 23.12)	11.82 ± 3.53* (7.05 - 21.17)

***. P< 0.001, ** P< 0.01, * 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 -aminolevulinic acid; PBG-U, Urinary porphobilonogen.

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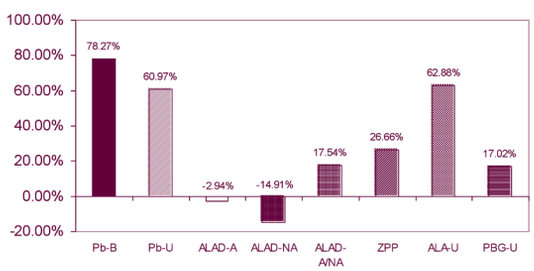

Figure 1.0: Percentage change of lead concentration in blood (Pb-B), lead concentration in urine (Pb-U), activated erythrocyte, δ-Aminolevulinic acid dehydratase (ALAD-Act) non-activated erythrocyte δ-Aminolevulinic acid dehydratase (ALAD-NA), activated and non-activated erythrocyte - δ Aminolevulinic acid dehydratase ratio (ALAD-A/NA), Erythrocyte- Zinc Protoporphyrin (ZPP), δ-amino levulinic acid in urine (ALA-U) and Porphobilinogen in urine (PBG-U) of SP group with respect to control group

ceruloplasmin was measured by method of Ravin (1961). Ceruloplasmin oxidizes P-phenylenediamine in the presence of oxygen to form a purple-coloured oxidised product. The cerulolasmin concentration was determined from the rate of oxidation of phenylendamine at 370C and at pH 6.0 which has an absorption peak at 530 nm.

Statistical analysis between control and SP group were carried out by students' 't' test. Pearson's correlation equation was also calculated to evaluate correlation between various parameters in control and SP groups separately.

Results

Table 1.0 summarises lead concentration in blood (Pb-B), lead concentration in urine (Pb-U), both activated and non-activated erythrocyte–ALAD and its ratio, erythrocyte ZPP, ALA in urine (ALA-U) and PB-G in urine (PBG-U) of occupationally lead-exposed spray painters (SP) group and control group. The Pb-B level of control group ranged from 2.8-22.0 microgram/dL whereas in the case of SP group it was 7.5-45.7microgram/dL. The Pb-B ($p < 0.001$) and Pb-U ($p < 0.01$) levels were significantly increased in SP group as compared to the control group. Activated, non-activated erythrocyte–ALAD activities were not significantly altered in SP group as compared to control group. However, the ratio of activated/non-activated erythrocyte ALAD activities showed a significant increase ($p<0.001$) in SP group as compared to the control group. Erythrocyte–ZPP level was not significantly changed in SP group as compared to the control group. Both urinary ALA (ALA-U) and PBG (PBG-U) of SP group showed a significant elevation ($p<0.001$ and $p<0.05$) respectively, compared to control group.

Figure 1.0 shows the percentage change of blood lead concentration (Pb-B), urinary lead concentration (Pb-U), ratio of activated/non-activated erythrocyte –ALAD, ALA in urine (ALA-U) in lead-exposed SP group with the unexposed control group. Figure 1.0 shows that blood lead concentration of SP group is increased by 78.27% compared with the control group. This was confirmed with a greater urinary lead excretion (60.97%) in the SP group. The SP group also showed an increase of activated/non-activated erythrocyte ALAD ratio (17.54%), erythrocyte ZPP (26.66%), PBG in urine (17.02%) and ALA in urine (62.88%) as compared to respective controls.

Figure 2.0 depicts a positive correlation ($r= 0.73$, $p<0.001$) between the concentration of blood lead (Pb-B) and urinary lead (Pb-U) of lead exposed SP group (Pb-B levels of 7.5-45.7 microgram/dL). But no correlation ($r = 0.29$) between blood lead concentration (Pb-B) and urinary lead (Pb-U) of the control group (Pb-B levels of 2.8 – 22.0 g/dL) was observed (Figure 3.0).

Figure 4.0 shows a positive correlation ($r = 0.45$, $p<0.01$) between blood lead concentration (Pb-B) and urinary ALA of spray painters (Pb-B levels of 7.5-45.7 microgram/dL). But no correlation ($r =0.02$) between blood lead concentration (Pb-B) and ALA in urine (ALA-U) of control group (Pb-B levels of 2.8 – 22.0 g/dL) was observed (Figure 5.0).

Table 2.0 shows hematological parameters of SP group and control group. No significant change in hematological parameters was observed as compared to control group.

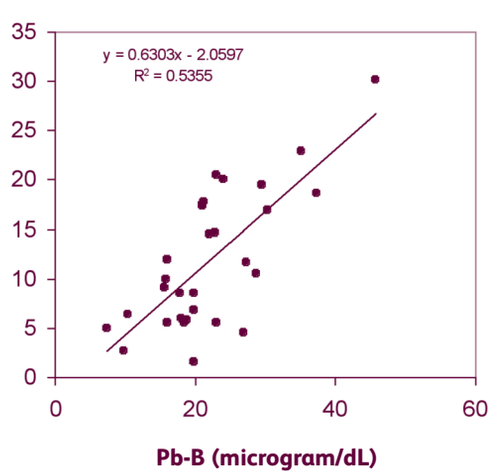

Figure 2.0: Relationship between blood lead concentration (Pb-B; range is 7.5 – 45.7 µg/dl) and lead concentration in urine (Pb-U) of SP group; n = 30, Correlation coefficient (r) is 0.73, $p <0.001$; $y = -0.6303x – 2.0597$

Groups	Hb gm/dL	PCV %	MCV (fl)	MCH (pg)	MCHC (g/dl)	Total Erythrocyte count (milllion/µL)	Total leucocyte count (/cu. mm)
Control groups (N-35)	14.36 ± 0.92 (12.6 - 16.3)	45.19 ± 2.29 (40.4-50)	88.77 ± 5.05 (76.7-109.7)	29.73 ± 2.05 (26.1- 36.6)	31.89 ± 0.89 (29.3 - 33.3)	5.10 ± 0.43 (4.26 - 5.71)	6.34 ± 1.66 (4.2 - 8.9)
Spray painters (N-30)	14.18 ± 1.23# (11.7-16.5)	44.09 ± 3.056# (36.8 - 51.2)	89.49 ± 4.25# (67.8 - 104)	29.62 ± 1.65# (20.3 - 32.5)	32.41 ± 0.95*** (29.5 - 34.4)	5.02 ± 0.57# (4.27 - 6.21)	6.89 ± 1.19# (6.1- 9.7)

*** P< 0.05, # Non-significant as compared with control group. Hb, haemoglobin concentration;PCV,Packed cell volume;MCV, mean corpuscular volume; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular haemoglobin concentration.

Table 3.0 depicts the serum MDA content, erythrocyte-SOD, erythrocyte-catalase activities and plasma ceruloplasmin in SP group and control group. The lipid peroxide level was significantly increased ($p<0.001$) and the antioxidant enzymes such as erythrocyte-SOD activities ($p<0.001$) and erythrocyte-catalase activities ($p<0.05$) were significantly decreased in the SP group as compared to the control group. However, no significant alteration of plasma ceruloplasmin levels was found in the SP group as compared to control group. Figure 6.0 depicts the percentage change of serum lipid peroxide (MDA), erythrocyte-SOD, erythrocyte-catalase activities and plasma ceruloplasmin in the SP group as compared to control group. Serum MDA level increased by 48.0% in the SP group as compared to the control. Erythrocyte-SOD, catalase activities and plasma ceruloplasmin in the SP group showed a decrease of 27.37%, 20.93% and 7.91% respectively from the control group.

Discussion

Blood lead (Pb-B) and urinary lead (Pb-U) levels significantly increased in the spray painters as compared to normal control subjects, which indicates a greater lead (Pb) absorption in spray painters group as compared to the control group. Lead absorption generally results in rapid urinary lead excretion. Greater lead exposure causes higher accumulation in bone followed by soft tissues. The Pb-B level not only depends on the equilibrium between absorption, storage and excretion but also on the acute (current) exposure as the short half life for lead in blood (28-36 days) is also influenced by previous storage. Blood lead level is the best and most sensitive biomarker for identifying lead pollution, human exposure and its adverse effects. However, single Pb-B measurements do not reflect the body burden of lead (ATSDR, 2005). The urine lead (Pb-U) excretion has also been considered as an index of exposure. Since Pb-B values change more rapidly than urine lead excretion, estimation of Pb-U appears to be of limited use for general screening, because various factors influence the urinary excretion of lead such as renal function, fluid

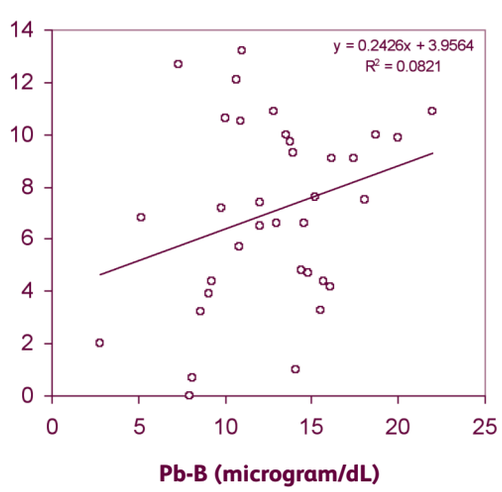

Figure 3.0: Relationship between blood lead concentration (Pb-B; range is 2.8 -22.0 δg/dl) and lead concentration in urine (Pb-U) of control group; n = 35, Correlation coefficient (r) is 0.29, Not significant; y = 0.2426x + 3.9564

Table 3.0: Lipid peroxide (MDA), superoxide dismutase (SOD) catalase, ceruloplasmin activities in spray painters and control group

Groups	Malondialdehyde (nmol/mL)	SOD (unit/mL of hemolysate)	MCV (fl)	MCH (pg)
Control group (N=35)	1.12 ± 0.480 (0.55 - 2.22)	14.12 ± 3.39 (4.57 - 19.42)	47.63 ± 17.94 (16.90-97.18)	53.17 ± 16.03 (11.37-83.12)
Spray painter (N=30)	1.658 ± 0.471*** (1.11 - 2.961)	10.24 ± 3.41*** (3.42 -17.14)	37.66 ± 9.46* (23.23-63.38)	48.96 ± 7.57# (33.25-64.75)

***. P< 0.001, * P< 0.05, # Non-significant as compared with control group

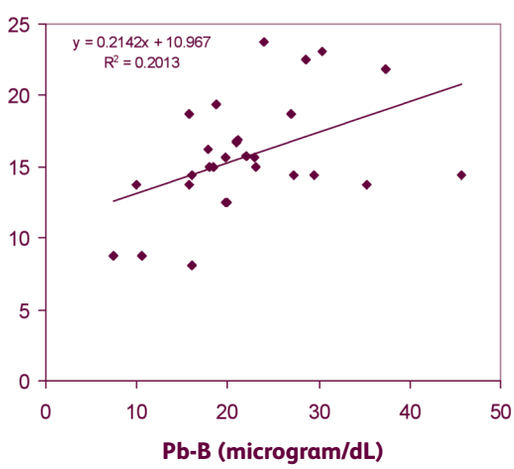

Figure 4.0: Relationship between blood lead concentration (Pb-B; range is 7.5 – 45.7 μg/dl) and - δaminolevulinic acid in urine (ALA-U) of SP group; n = 30, Correlation coefficient (r) is 0.45, p< 0.01; y = 0.2142x + 10.967

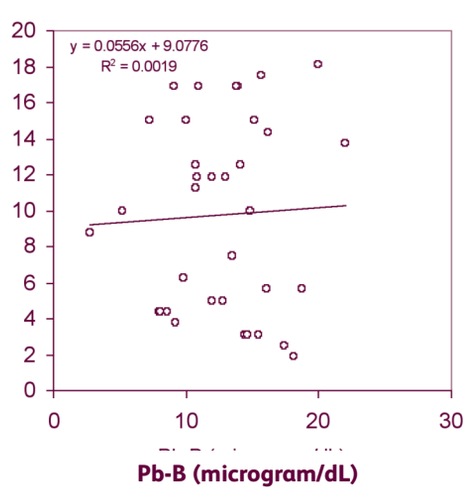

Figure 5.0: Relationship between blood lead concentration (Pb-B; range is 2.8 – 22.0 g/dl) and - aminolevulinic acid in urine(ALA-U) of control group; n = 35, Correlation coefficient (r) is 0.04, p> 1.0, Not Significant; y = 0.0556x + 9.0776

intake and the specific gravity of the urine (Ellis, 1966). But Pb-U levels after administration of the chelating agent like CaNa2 EDTA is considered to be an excellent measure of the potentially toxic fraction of the total body burden of lead (ATSDR, 2005).

In our study the Pb-B value range in spray painters (7.5 to 45.7microgram/dL) and the control group (2.8 to 22 microgram/dL) in association with the Pb-U value range in spray painters (1.5 to 30.2 microgram/dl) and control group (1 to 13.2 microgram/dL) indicate a greater absorption of lead in case of spray painter group as compared to control group. A positive correlation between Pb-B and Pb-U (Figure 2.0) in spray painters group and no correlation in control group (Figures 2.0 and 3.0) corroborate the above statement. Although lead free or low lead content paints are now available in-market, lead is still used in various paints because of its anticorrosive properties. The d-ALAD (E.C 4.2.1.2.4) catalyses condensation of two molecules of d-ALA to form the mono-pyrrole, PBG. The d-ALAD is a zinc dependent metalloenzyme and zinc partly protects this enzyme against the adverse effects of lead in vitro (Tsukamoto *et al.,* 1979) and possibly also in vivo (Cerklewski,1976). The d-ALAD enzyme is activated by Zn-acetate. The non-significant alteration of activated and non-activated d-ALAD level in spray painters group indicates a relative lower range of Pb-B level (7.5 to 45.7microgram/dL). Several studies reported that the d-ALAD activity is usually reduced to 50% of normal activity when Pb-B values are in the range of 30-50 microgram/dl (WHO, 1995; ATSDR,2005). However, the ratio of activated to non-activated d-ALAD was increased significantly in the

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SP group as compared to the control group. This indicates the inhibition of non-activated d-ALAD ($p < 0.001$) by the lead. This is further supported by increased excretion of d-ALA in urine in spray painters as compared to the control group. The decreased activity of d-ALAD in our study is associated with increased urinary excretion of d-ALA. A positive correlation between Pb-B and urinary d-ALA in spray painters (Figure 4.0) and no correlation in control group (Figure 5.0) clearly indicates a greater rate of urinary excretion of d-ALA in higher range of Pb-B level (7.5 to 45.7microgram/dL).

The RBC-Zinc protoporphyrin (ZPP) concentration was not altered in the spray painters group (10.45±3.35 microgram/dL) as compared to the control group (8.25±7.79 microgram/dL). The reason may be a relatively lower range of blood lead level (Pb-B -7.5 to 45.7 microgram/dL) in the spray painters group. The ZPP level was elevated in about 50-75% of those persons who have the range of Pb-B levels (40-60 microgram/dL) without any symptoms and are almost always elevated in symptomic lead poisoning (WHO, 1995; ATSDR, 2005).

In the spray painters group, all hematological parameters except MCHC were statistically non significant as compared to normal control. Lead affects the hematopoietic system and decrease the Hb synthesis but this occurs only with higher levels of lead exposure. Past reports and this present result indicate that anemia is not common at low Pb-B level in spray painters. Therefore the determination of hematological parameters appear to be not convenient for screening the low lead exposure such as the spray painters group.

The present study finds a significant increase of erythrocyte lipid peroxide level ($p<0.001$) and significant decrease in the activities of erythrocyte antioxidant enzymes such as superoxide dismutase ($p<0.001$) and catalase ($p<0.05$) in the spray painters group as compared to the control group but no significant alteration in plasma ceruloplasmin was observed in the spray painter group. Lead induced oxidative stress is not completely understood. Lead may cause oxidative stress by accelerating prooxidants formation and reducing the antioxidant defense of cells or by inducing both. Lead is known to have some toxic effects on membrane function (Donaldson *et al.,* 1996). It alters the RBC membrane, since RBCs have high affinity for lead and majority of the lead is found in RBCs; hence RBCs are more vulnerable to oxidative damage than any other cells (De silva,1981;

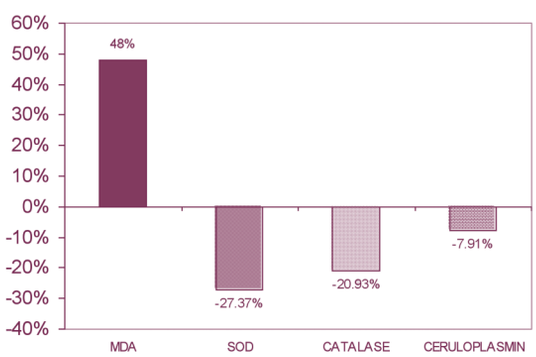

Figure 6.0: Percentage change chart of serum malondialdehyde (MDA), erythrocyte superoxide dismutase (SOD), erythrocyte catalase and plasma ceruloplasmin of SP group with respect to control group

Rice-Evans, 1990; Leggett, 1993,). Lead toxicity increases osmotic and mechanical susceptibilities of RBC is reported in several studies (Waldron, 1966). The activity of some membrane bound enzymes (Bonting *et al.*, 1963; Hasan *et al.,* 1971, Raghavan, 1981) and composition of membrane proteins (Fukumoto *et al.,* 1983) in RBC were also found to be altered by lead exposure. The interaction of heavy metals with oxy-haemoglobin has already been suggested as an important source of superoxide formation in RBC (Curell, 1975; Das *et al.,* 2006).

Decreased SOD and catalase activities in the spray painters group indicate possible lead-induced generation of O2.- and H2O2. Thus it is speculated that Pb2+ may induce a generation of ROS by interacting with oxy-haemoglobin, which may lead to per-oxidative damage of RBC membrane. The accumulated d-ALA induces ROS generation is reported in several studies (Monteriro, *et al.,* 1989; *Monteriro et al.,* 1991; Hermes Lima *et al.,* 1991). In the present study, we found that the excretion of d-ALA in urine was significantly high as compared to controls. It indicates that the accumulation of d-ALA was more in cells.

Now a question arises: how can accumulated d-ALA induce ROS generation? It is possible that d-ALA may undergo enolisation and autoxidation at pH 7.0-8.0 resulting in generation of superoxide anions. Also d-ALA/oxy-haemicrogramlobin-coupled oxidation may result in ROS generation. Superoxide (O2.-) which were generated owing to d-ALA, d-ALA/Oxy Hb-coupled autoxidation (Moneterio, 1986) can interact and generate hydroxyl radicals (OH-), which is considered to be the highest reactive among ROS. In the present study, the decreased erythrocyte- SOD and catalase activities in the spray painters group suggest the generation of O2.-

and H2O2 respectively and also inhibition of d-ALA/Oxy Hb coupled autoxidation. The decreased SOD activity in this study group is probably related to the interaction of lead with copper, since SOD is a 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 (Mylroie *et al.,* 1984; Mylroie *et al.,* 1986; Adler *et al.,* 1993). Lead might have caused adverse effects in the spray painters by this mechanism that builds up the ROS and increases oxidative stress. 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 *et al.,* 2001; Das and Das, 2004). Hence it is not clear whether these alterations are the cause of the oxidative damage or its effects on the genetic materials are responsible for the synthesis of endogenous antioxidant enzyme system of cells. It is possible that initially lead may affect some components of the cellular antioxidant defence system and that might cause impairment in pro-oxidant/antioxidant balance in cells resulting in oxidative damage (Moneterio *et al.,* 1985; Ito *et al.,* 1985; Sugawara *et al.,* 1991).

Conclusions

Therefore, the study clearly showed an adverse effect on heme biosynthesis and imbalance of pro-oxidant/antioxidant status of spray painters from Western Maharashtra (India) by increasing lipid peroxidation in association with decrease erythrocyte-SOD and catalase activities. The protection to the workers from the health hazards of occupational lead exposure is essential. The potential risk of lead toxicity will persist unless safety measures are taken by the employers and monitored by social groups and government agencies.

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