Ind J Clin Biochem (Oct-Dec 2011) 26(4):400–406
DOI 10.1007/s12291-011-0159-6

ORIGINAL ARTICLE

Biochemical Effects of Lead Exposure on Systolic & Diastolic Blood Pressure, Heme Biosynthesis and Hematological Parameters in Automobile Workers of North Karnataka (India)

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Received: 7 February 2011 / Accepted: 15 August 2011 / Published online: 21 September 2011

Abstract The purpose of this study was to find out the effect of lead exposure on systolic and diastolic blood pressure, heme biosynthesis related and hematological parameters of automobile workers. For this study 30 automobile workers were selected and compared with 30 age matched healthy control subjects. Significantly increased blood lead (364%, $P < 0.001$) and urinary lead (176%, $P < 0.001$) levels were observed in automobile workers (study group) as compared to controls. Systolic blood pressure (5.32%, $P < 0.05$) and diastolic blood pressure (5.87%, $P < 0.05$) were significantly increased in the automobile workers as compared to controls. The significantly decreased non-activated erythrocyte δ-aminolevulinic acid dehydratase (δ-ALAD) (−18.51%, $P < 0.01$) and activated δ-ALAD (−13.29%, $P < 0.05$) levels were observed in automobile workers as compared to normal healthy control subjects. But the ratio of activated/non-activated δ-ALAD was significantly increased (43.83%, $P < 0.001$) in automobile workers as compared to controls. Excretions of δ-aminolevulinic acid (83.78%, $P < 0.001$) and porphobilinogen (37%, $P < 0.001$) in urine were significantly increased in the study group as compared to the controls. In automobile workers heamoglobin (−11.51%, $P < 0.001$), hematocrit (−4.06%, $P < 0.05$), mean corpuscle volume (−3.34%, $P < 0.05$), mean corpuscle hemoglobin (−5.66%, $P < 0.01$), mean corpuscle hemoglobin concentration (−7.67%, $P < 0.001$), red blood cell count (−14.6%, $P < 0.001$) were significantly decreased and total white blood cell count (11.44%, $P < 0.05$) increased as compared to the controls. The results of this study clearly indicate that the absorption of lead is more in automobile workers and it affects on blood pressure, heme biosynthesis and hematological parameters observed in this study group.

Keywords Automobile workers · Blood lead (Pb-B) · Urinary lead (Pb-U) · Systolic and diastolic blood pressure · δ-Aminolevulinic acid (δ-ALA) · δ-Aminolevulinic acid dehydratase (δ-ALAD) · δ-Aminolevulinic acid synthetase (δ-ALAS) · Porphobilinogen (PBG) · Hematological parameters

Introduction

Lead is one of the most widely scattered toxic metals in the world and used by mankind for over 9,000 years. Lead in the environment may be derived from natural or anthropogenic sources. Lead and its compounds 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 [1–5].

Lead is absorbed by the gastrointestinal tract via food, beverages, soil and dust. Dietary factors, nutritional status,

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chemical form of the metal and patterns of food intake affect lead absorption. In humans, lead causes a wide range of biological effects depending upon the level and duration of exposure. It affects several organs and organ systems including nervous, renal, reproductive, hematological and immune system [2, 6–10]. Lead also affects cardiovascular system and increases systolic and diastolic blood pressure [11].

Lead interferes with heme biosynthesis by altering the activity of three enzymes δ-aminolevulinic acid synthetase (δ-ALAS), δ-aminolevulinic acid dehydratase (δ-ALAD) and ferrochelatase. Also lead affects the hematological system. The anaemia induced by lead is microcytic and hypochromic and results primarily from both inhibition of heme and globin synthesis and shortening of the erythrocyte lifespan [12, 13].

Adverse biochemical effects of lead are well known today. A correlation between clinical signs and symptoms with blood lead level and relevant biochemical changes may provide important information, for making suitable changes in the working environment of occupationally lead exposed workers. Hence this study was carried out to find out the adverse effects of occupational lead exposure on blood pressure, heme biosynthesis related parameters and hematological parameters in automobile workers of North Karnataka, India.

Materials and Methods

The study was carried out in 30 automobile workers with occupational exposure to lead (Study group) from Bijapur (North Karnataka), India, and same age matched 30 normal healthy, non-occupational lead exposure subjects were taken as controls from the same place for comparison. All the study and control group subjects had age in the range of 20–45 years.

Prior to data and biological specimen collection, workers were informed on the study objectives and health hazards of lead exposure. Informed consent was obtained from all workers. Demographic, occupational and clinical data were collected by using questionnaire and interview.

Most of the automobile workers had major complaints of muscle pains, itchy feeling, mild fatigue, aggressiveness, irritability, lethargy, poor concentration, abdominal discomfort, etc. None of the subjects had a past history of major illness. Dietary intake and food habits of all subjects were normal. Non-smokers, non-alcoholic healthy males, who were occupationally exposed to lead for more than 6 h per day with the duration of exposure from 2 to 20 years, were selected for this study. Most of the workers consumed mixed type of diet. The entire protocol was approved by the institutional ethical committee.

Blood pressure was measured in supine position i.e. resting position of the workers prior to blood collection with the help of sphygmomanometer. Systolic and diastolic blood pressure was expressed as mm/Hg.

Blood was collected by venipuncture into EDTA tubes. At the same time of blood collection, random urine samples were collected to avoid errors from inadequate collection of 24 h urine sample from each subject into dark brown and amber colored bottles.

Estimations of lead in blood and urine were carried out by graphite farness atomic absorption spectrophotometer (AAS) using a Perkin Elmer model 303 fitted with a boiling 3 slot burner. The AAS was connected to Hitachy 165 recorder and values were shown in microgram per liter [14].

Erythrocyte delta-ALAD was estimated by the Julian Chisolm method [15, 16]. Erythrocyte delta-ALAD acts on aminolevulinic acid (ALA) to form porphobilinogen (PBG), which is further reacted with modified Ehrlich's reagent to form a pink-coloured compound measured on a spectrophotometer at 555 nm. Hg-TCA stops the reaction by precipitating the proteins. ALAD activity is estimated using the formula:

$$\delta\text{-}ALAD\ activity = \frac{Net\ absorbance \times 100 \times 2 \times 35}{\%hematocrit \times 60 \times 0.062} \times (1\mu\text{mol}\delta\text{ - ALA utilized})/\text{min}/\text{l of erythrocytes})$$

where 2 is the conversion factor for δ- ALA to PBG, 35 is the dilution factor, 60 is the incubation time (min), 0.062 is the micromolar absorptivity of modified Ehrlich's reagent and PBG chromogen.

Erythrocyte δ-ALAD activated by zinc acetate and ratio of activated/non-activated δ-ALAD (Act/Non-act) was calculated.

Urinary δ-ALA was estimated by Osamu et al. method [17]. δ-ALA reacts with acetyl acetone and forms pyrrole substance, which reacts with *p*-dimethylaminobenzaldehyde. The colored complex was measured spectrophotometrically at 555 nm. The results were expressed as mg/l.

Phorphobilinogen in urine was estimated by the Mauzerall and Granick method [18]. PBG from urine reacts with *p*-dimethylaminobenzaldehyde (DMAB, Ehrlich's reagent) in acid solution to form a red compound which is measured at 555 nm exactly after 5 min; the values were calculated according to the Rimington formula [19].

$$Urinary\ PBG(\text{g/l}) = \frac{O.D \times Number\ of\ times\ the\ urine\ diluted}{70.85}$$

All hematological parameters were measured using a fully automated Hematology analyzer Sysmax K-4500 [20]. Statistical analyses between the control and study group were done using the unpaired student's *t*-test.

P values less than 0.05 were considered statically significant.

Results

Table 1 summarizes the mean values of PbB, PbU and haeme biosynthesis related parameters in automobile workers and control group and Table 2 summarizes mean values of hematological parameters of automobile workers and unexposed Control group. Figure 1 Shows the Percentage change of PbB, PbU and haeme biosynthesis related parameters in automobile workers with respect to control group. Figure 2 Shows the Percentage change of mean values of hematological parameters of automobile workers with respect to control group and Figure 3 shows the mean values of Systolic and Diastolic blood pressure of the Control and Automobile workers.

Discussion

Blood lead (364%, $P < 0.001$) and urinary lead (176%, $P < 0.001$) levels were significantly increased in automobile workers as compared to controls (Table 1; Fig. 1) indicates more absorption of lead. Lead absorption results in its rapid urinary excretion. Blood lead levels generally reflect acute (current) exposure because of short half life of lead in blood (28–36 days), Estimation of blood lead is the best and most sensitive biomarker for identifying lead pollution, human exposure and its adverse effects [1].

Automobile workers who were involved mainly in spray painting, radiator and battery repairs were selected for this study. Earlier lead was used in paints due to its anticorrosive property. Since 1984 addition of lead in paints has been banned in almost all countries in the world, but still small amount of lead is present in colour pigments as reported in several studies [21]. Lead is used for soldering of leaking radiators. It is also used in making lead plates and grids of batteries. Nowadays the automobile sector is rapidly growing and many automobile workers are exposed to lead through their routine activities like spray painting, radiator and battery repairs. While working, these workers did not take proper precautions as they did not use protective masks, hand gloves as well as special apron. Also they did not wash their hands before taking their food and majority of workers took their lunch in the garages. Increased blood lead level in these workers indicates that the release of lead fumes, particles, dust and vapours were more in those places. Poor hygiene and inappropriate protection increases the risk of exposure.

The estimation of urinary lead has also been employed as an index of exposure. The spot random urine sample collection is also suitable for screening lead exposed population as the collection of 24 h urine sample is inconvenient. Urinary lead reflects blood lead fairly well. However it is not as suitable because various factors other than the degree of lead absorption alone influence the excretion of lead; such as renal function, fluid intake and the specific gravity of the urine [22].

We have activated δ-ALAD by adding Zn-acetate and estimated the activity of activated δ-ALAD, nonactivated δ-ALAD and the ratio of activated δ-ALAD/nonactivated δ-ALAD and found non-activated erythrocyte δ-ALAD levels were significantly decreased (−18.5%, $P < 0.01$) in automobile workers as compared to the control subjects. The activated δ-ALAD mean values were significantly decreased (−13.29%, $P < 0.05$) but the ratio of activated/non-activated δ-ALAD was significantly increased (43.83%, $P < 0.001$) in automobile workers as compared to controls. (Table 1; Fig. 1). These results indicate that lead inhibits the activity of δ-ALAD enzyme in these workers.

Table 1 Mean values of PbB, PbU and haeme biosynthesis related parameters in automobile workers and control group

Sl no	Biochemical parameters	Control group ($N = 30$)	Automobile workers ($N = 30$)
1	PbB (μg/dl)	10.2 ± 5.8 (2.0–23.0)	47.37 ± 23.22*** (5.0–85.0)
2	PbU (μg/dl)	6.28 ± 3.83 (1.0–14.0)	17.37 ± 12.5*** (1.0–41.0)
Haeme biosynthesis related parameters			
Erythrocyte δ-ALAD activity unit expressed at Sl. no. 3 and 4 (μmol δ-ALA utilized/min/liter of erythrocytes)			
3	Activated-δ-ALAD	19.70 ± 4.96 (4.73–28.62)	17.08 ± 3.75* (14.81–24.50)
4	Non-activated-δ-ALAD	16.31 ± 4.54 (4.03–32.70)	13.29 ± 4.74** (3.46–28.39)
5	Ratio of Act/N-Act δ-ALAD	1.46 ± 0.83 (0.42–2.28)	2.10 ± 0.99*** (1.27–4.05)
6	U-δ-ALA (mg/l)	9.62 ± 5.45 (2.5–17.5)	17.68 ± 4.42*** (4.69–27.94)
7	U-PBG (mg/l)	10.10 ± 2.87 (3.5–15.87)	13.84 ± 3.3*** (8.47–18.76)

Figures without parenthesis indicate Mean ± SD values and those in parenthesis are range of values of the present study groups

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$ as compared to controls

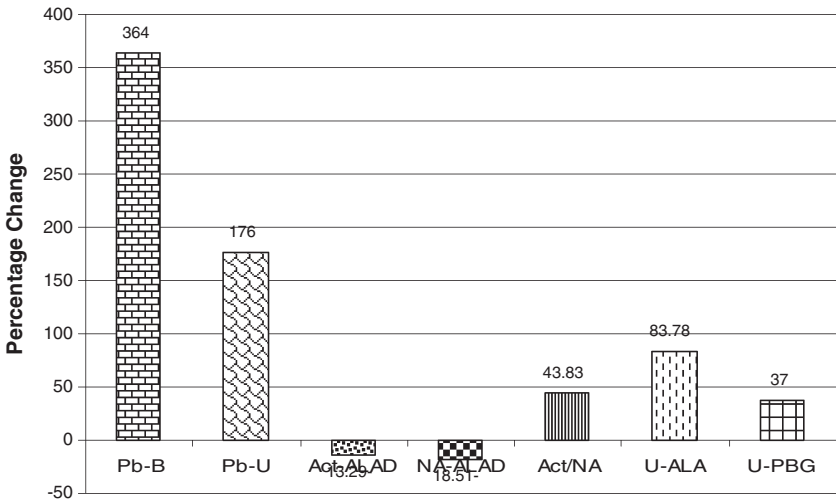

Fig. 1 Percentage change of Pb-B, Pb-U and haeme biosynthesis related parameters in automobile workers with respect to control group. *Pb-B* blood lead, *Pb-U* urinary lead, *Act-ALAD* activated δ-aminolevulinic acid dehydratase, *NA-ALAD* non-activated δ-aminolevulinic acid dehydratase, *Act/NA* activated δ-aminolevulinic acid dehydratase/non-activated δ-aminolevulinic acid dehydratase, *U-ALA* urinary δ-aminolevulinic acid and *U-PBG* urinary phorphobilinogen

δ-ALAD (E.C.4.2.1.24) catalyses condensation of two molecules of δ-ALA to form the mono-pyrrole PBG. δ-ALAD is a zinc-dependent metalloenzyme and zinc partly protects this enzyme against the adverse effect of lead in vitro and also in vivo [22]. δ-ALAD activity decreased due to lead can be reversed by adding Zn or dithiothreitol (DTT) in vitro [23]. Possible mechanism of reactivation includes reduction of sulfhydryl groups, which are essential for enzyme activity, or, in the case of DTT, chelation of lead from binding sites on the enzyme. Exposure to lead does not decrease the concentration of δ-ALAD in erythrocytes, but substantially decreases δ-ALAD activity [23].

Non-activated δ-ALAD activity alone is considered as a predictor of Pb-B concentration, as in the European standardized and other similar δ-ALAD assay methods [23]. The activated/non-activated δ-ALAD activity ratio is a good marker for lead toxicity. In this study δ-ALAD was activated by using zinc acetate and the activated, non-activated δ-ALAD activity values were measured. The ratio of activated/non-activated δ-ALAD was calculated. It is observed that the ratio of activated/non-activated δ-ALAD was significantly increased (43.83%, $P < 0.001$) in automobile workers as compared to the controls. It confirms that the δ-ALAD activity was decreased or inhibited by the lead in the automobile workers as compared to control group. Measurement of δ-ALAD activity in the erythrocytes offers a good and simple method of evaluation of lead poisoning (Table 1; Fig. 1).

Excretions of δ-ALA (83.78%, $P < 0.001$) and PBG (37%, $P < 0.001$) in urine were significantly increased in automobile workers as compared to the controls (Table 1; Fig. 1). These results indicate that there is inhibition of the enzymes of the heme biosynthetic pathway resulting in the accumulation and increased excretion of the intermediates in the heme biosynthetic pathway, namely, δ-ALA and PBG. Lead interferes with the biosynthesis of heme by altering the activity of three enzymes δ-ALAS, δ-ALAD and Ferrochelatase. Lead indirectly stimulates the mitochondrial enzyme δ-ALAS which catalyzes the condensation of glycine and succinyl CoA to form ALA. The δ-ALAS catalyzed reaction is the rate limiting step in heme biosynthesis; increase of δ-ALAS activity occurs through feedback derepression. Lead inhibits the zinc containing cytosolic enzyme δ-ALAD which catalyzes the condensation of two units of δ-ALA to form PBG. This inhibition is non competitive and occurs through the binding of active site of δ-ALAD. Lead bridges the vicinal sulfhydryl, where as zinc, which is normally found at the active site, binds to only one of these sulfhydryl. Inhibition of δ-ALAD and feedback derepression of δ-ALAS results in accumulation of δ-ALA. Estimations of urinary δ-ALA and PBG are also useful markers for screening lead exposed workers [24].

The immediate effect of the inhibition of δ-ALAD will be an increased level of δ-ALA in the blood, which will then lead to its increased excretion in urine. The plasma levels of δ-ALA are elevated in the presence of higher lead levels [24]. Thus, it appears that lead has discernable effects on the urine levels of δ-ALA. Therefore, estimations of urinary δ-ALA and PBG are good indicators of body lead burden.

In automobile workers the levels of heamoglobin (Hb) (−11.51%, $P < 0.001$), hematocrit (−4.06%, $P < 0.05$), mean corpuscle volume (MCV) (−3.34, $P < 0.05$), mean corpuscle hemoglobin (MCH) (−5.66%, $P < 0.01$), mean corpuscle hemoglobin concentration (MCHC) (−7.67%, $P < 0.001$), red blood cell (RBC) count (−14.6%, $P < 0.001$) were significantly decreased and total white blood cell (WBC) count (11.44%, $P < 0.05$) was increased as compared to the controls (Table 2; Fig. 2).

Lead impairs the rate of incorporation of iron into mature and immature RBC in cases of human lead poisoning [25]. Lead affects the hematopoietic system and

Table 2 Mean values of hematological parameters of automobile workers and unexposed control group

Sl no	Hematological parameters	Control group $N = 30$	Automobile workers $N = 30$
1	Hb (gm/dl)	14.85 ± 1.46 (10.4–16.0)	13.14 ± 1.36*** (11.9–15.5)
2	Hct (%)	45.50 ± 3.80 (36.70–51.1)	43.65 ± 3.05* (37.50–57.50)
3	MCV (fl)	85.94 ± 5.29 (68–91.1)	83.10 ± 4.23* (78.20–91.8)
4	MCH (pg)	28.23 ± 2.44 (19.3–31.90)	26.63 ± 1.85** (23.9–31.40)
5	MCHC (gm/dl)	32.82 ± 1.44 (28.30–35.5)	30.30 ± 1.44*** (26.80–33.80)
6	RBC count (Million/μl)	5.89 ± 0.76 (4.11–7.74)	5.03 ± 0.515*** (4.69–7.30)
7	WBC count (cells/cumm)	6.73 ± 1.82 (5.2–9.3)	7.50 ± 1.39* (5.17–9.80)

Figures without parenthesis indicate Mean ± SD values and those in parenthesis are range of values of the present study groups

* $P < 0.05$; ** $P < 0.01$, *** $P < 0.001$ as compared to control

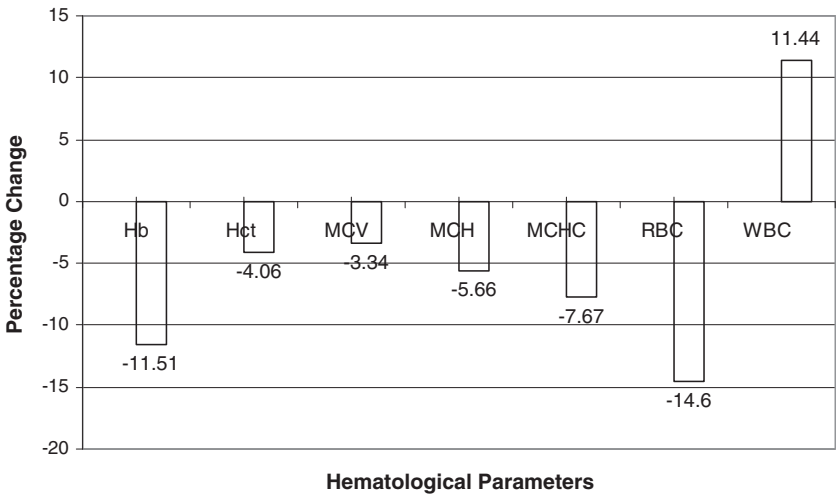

Fig. 2 Percentage change of mean values of hematological parameters of automobile workers with respect to control group. *Hb* heamoglobin, *Hct* hematocrit, *MCV* mean corpuscle volume, *MCH* mean corpuscle hemoglobin, *MCHC* mean corpuscle hemoglobin concentration, *RBC* red blood cell count, *WBC* white blood cell count

reduces the hemoglobin synthesis, but this occurs only with high levels of exposure. It might be due to decreased heme and globin synthesis or erythrocyte formation and function. Erythrocyte survival also decreases by lead due to inhibition of membrane bound Na^+-K^+-ATPase [26]. Erythrocyte formation is regulated by erythropoietin hormone and the serum level of this hormone is decreased by the lead [1].

Significantly decreased Hb, MCV, MCH, MCHC, RBC count in automobile workers may be due to decreased heme concentration or decreased erythropoietin hormone or decreased iron absorption or decreased maturation of RBC by lead. Significantly increased total WBC count in these automobile workers could be due to more exposure to dust or fumes of lead. Therefore, the estimation of hematological parameters is useful for screening the occupational lead exposed workers.

Lead is known to affect the cardiovascular system in occupationally lead exposed populations and also in experimental animals. The slightly increased systolic blood pressure (5.32%, $P < 0.05$) and diastolic blood pressure (5.87%, $P < 0.05$) in automobile workers with respect to controls indicate that increased blood lead does not alter blood pressure severely (Fig. 3). Intense and prolonged lead exposure has been attributed as a cause of hypertension. The observations of lead poisoning effects secondary to exposure to high levels showed increased incidence of strokes, kidney diseases and hypertension. It is reported that blood lead levels contribute independently to the increased systolic and diastolic blood pressure [27, 28]. The effect of lead exposure on hypertension is usually because of excessive occupational exposure and its effects on kidney functions. Kidney compromise and in turn effects on blood pressure. Lead has both direct and indirect effects on the blood vessel and the smooth muscle contractility and thereby affects blood pressure. Hypertension is prominent in workers known to be chronically exposed to lead. So it is possible that lead induced nephrotoxicity is a probable cause of secondary hypertension in these workers.

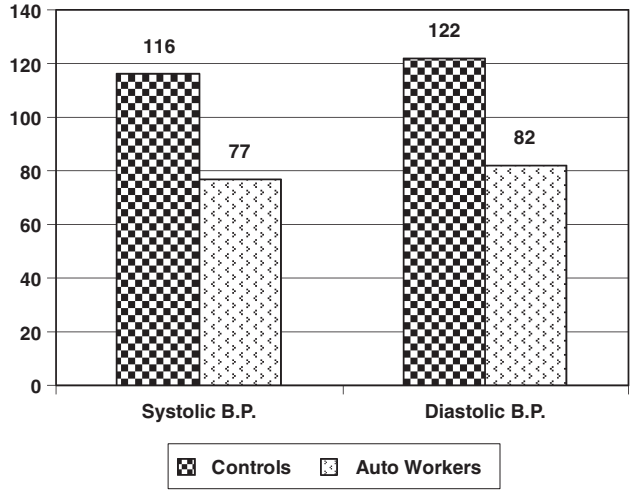

Fig. 3 Shows the mean values of systolic and diastolic blood pressure of the control and automobile workers

Conclusion

This study indicates that there is more absorption of lead in automobile workers, which results into increased excretion of lead in urine. Urinary lead excretion can be used as an index of exposure, since blood lead values change more rapidly than urinary lead. The estimation of δ-ALAD activity in erythrocyte is a very good, sensitive, most reliable, marker enzyme for screening the occupational lead exposure. Estimations of urinary δ-ALA and PBG are the good indicators of body lead burden further it is also indirectly useful to know the inhibition of δ-ALAD enzyme activity and PbB level in lead exposure population. This study also reveals that a complete hemogram, urinary ALA, PBG, Pb-B, Pb-U and erythrocyte delta-ALAD activities, measurements of blood pressure are useful in screening for occupational lead exposure. This study is useful to create awareness of health hazards related to lead exposure among occupationally lead exposed workers.

Acknowledgments We acknowledge the kind co-operation of the automobile workers and control subjects who volunteered for this study. Without them this work would not have been possible. We also acknowledge the facilities provided by the Dept. of Biochemistry, Dr. V. M. Govt. Medical College Sholapur, B.L.D.E.U's Shri B. M. Patil Medical College, Bijapur and common facility centre at Shivaji University, Kolhapur.

References

1. World Health Organization. Biological indices of lead exposure and body burden. In: IPCS, inorganic lead environmental health criteria 118, vol. 165. Geneva: WHO; 1995. p. 114–8.
2. Agency for Toxic Substances and Disease Registry (ATSDR). Toxicological profile for lead. Atlanta: US Department of Health and Human Services, US Government Printing; 2005. p. 102–225.
3. Liu J, Goyer RA, Waalkes MP. Toxic effects of metals. In: Klaassen CD, editor. Casarett and Doull's Toxicology: The basic science of poisons. 7th ed. USA: Mc Graw Hill Publication; 2008. p. 943–947.
4. Saryan LA, Zenz C. Lead and its compounds. In: Dickerson OB, Hrvarth EP. Jr, editors. Occupational Medicine. 3rd ed., USA: Mosby Publishing Company; 1994. p. 506–540.
5. IPSC Environmental Health Criteria 85. Lead-environmental effect. Geneva: WHO; 1989. p. 106.
6. Dongre NN, Suryakar AN, Patil AJ, Rathi DB. Biochemical effects of occupational lead exposure to workers in small scale automobile workshops of North Karnataka (India). J Env Health Res. 2010;10(01):27–34.
7. Patil AJ, Bhagwat VR, Patil JA, Dongre NN, Ambekar JG, Das KK. Biochemical aspects of lead exposure in silver jewellery workers of Western Maharashtra (India). J Basic Clin Physiol Pharmacol. 2006;17(4):213–29.
8. Can S, Baocy C, Ozaslan M, Bozkurt AY, Cengiz B, Cakmak EA, Kocaba R, Karadao E, Tarakcyoolu M. Occupational lead exposure effect on liver functions and biochemical parameters. Acta Physiol Hung. 2008;95(4):395–403.
9. Dresner DL. Modulation of bone marrow heme and protein synthesis by trace elements. Environ Res. 1982;28:55–66.
10. Dongre NN, Suryakar AN, Patil AJ, Rathi DB. Occupational lead exposure in automobile workers in North Karnataka (India): effect on liver and kidney functions. Al Ameen J Med Sci. 2010;3(4):284–92.
11. Vupputuri S, He J, Muntner P, Bazzano LA, Whelton PK, Batuman V. Blood lead level is associated with elevated blood pressure in blacks. Hypertension. 2003;41:463.
12. Verrula GR, Noah PK. Clinical manifestations of childhood lead poisoning. J Trop Med Hyg. 1990;93:170–7.
13. Papanikoulou NC, Hatzidaki ElG, Belivantis S, Tzanzkakis GN, Tsatsakis AM. Lead toxicity update. A brief review. Medical Sci Monit. 2005;11(10):RA329–36.
14. Parson PJ, Slavin W. A rapid Zeeman graphite furnace AAS method for determination of lead in blood. Spectrochim Acta. 1993;48B:925–39.
15. Grenik AA. Determination of lead chelated with EDTA in blood after precipitation of protein with perchloric Acid. Br J Ind Med. 1970;27:40–2.
16. Chisolm JJ Jr, Thomas DJ, Hamill TG. Estimation of δ-ALAD activity from erythrocytes. Clin Chem. 1985;31/4:601–5.
17. Schlick E, Mengel K, Friedberg KD. The effect of low doses in vitro and vivo on the δ-ALAD activity of erythrocytes bone marrow cells, liver and brain of the mouse. Arch Toxicol. 1983;53:193–205.
18. Wada O, Kohei T, Gumpei U, Yuzo Y, Kiku N. A simple method for the quantitative analysis of urinary δ-ALA to evaluate lead absorption. Br J Ind Med. 1969;26:240–3.
19. Mauzerall D, Granick S. The occurrence and determination of delta-ALA and PBG in urine. J Biol Chem. 1956;219:435–46.
20. Trester C. Modern technology for cellular differentiation: principles of measurement in Sysmex hematology analyzer technology. Paper presented at update in laboratory Haematology Sysmex Scientific Seminar, 10th Oct 1999.
21. Chem J-D, Wang JD, Jang JP, Chem YY. Exposure to mixtures of solvents among paint workers and biochemical alterations of liver function. Br J Ind Med. 1991;48:696–707.
22. Ellis RW. Urinary screening tests to detect excessive lead absorption. Br J Ind Med. 1966;23:263–75.
23. Sakai T, Yanagihara S, Ushio K. Restoration of lead inhibited δ-ALAD activity in whole blood by heat, zinc ion and (or) dithiotheritol. Clin Chem. 1980;26:625–8.

24. Patil AJ, Bhagwat VR, Patil JA, Dongre NN, Ambekar JG, Das KK. Biochemical aspects of lead exposure and toxicity in spray painters of western Maharashtra (India). J Environ Health Res. 2007;6(2):43–52.
25. Meredith PA, Moore MR, Campbell BC, Thompson GG, Goldberg A. δ-ALA metabolism in normal and lead-exposed humans. Toxicology. 1978;9:1–9.
26. O'Flaherty EJ, Hammond PB, Lerner SI, Hanenson IB, Roda SMB. The renal handling of δ-ALA in the rat and in the human. Toxicol Appl Pharmacol. 1980;55:423–32.
27. Rhagavan SRV. Erythrocyte lead-binding protein after occupational exposure II, influence of lead inhibition of membrane Na^+ -K^+- ATPase. J Toxicol Environ Health. 1981;7:561–8.
28. Perazella MA. Lead and the kidney: nephropathy, hypertension, and gout. Am J Dis Child. 1991;145:681–715.