

Status of Serum Calcium, Vitamin D and Parathyroid Hormone and Hematological Indices Among Lead Exposed Jewelry Workers in Dhaka, Bangladesh

I. Mazumdar, K. Goswami & Md Suhrab Ali

**Indian Journal of Clinical
Biochemistry**

ISSN 0970-1915
Volume 32
Number 1

Ind J Clin Biochem (2017) 32:110-116
DOI 10.1007/s12291-016-0582-9



Your article is protected by copyright and all rights are held exclusively by Association of Clinical Biochemists of India. This e-offprint is for personal use only and shall not be self-archived in electronic repositories. If you wish to self-archive your article, please use the accepted manuscript version for posting on your own website. You may further deposit the accepted manuscript version in any repository, provided it is only made publicly available 12 months after official publication or later and provided acknowledgement is given to the original source of publication and a link is inserted to the published article on Springer's website. The link must be accompanied by the following text: "The final publication is available at link.springer.com".

Status of Serum Calcium, Vitamin D and Parathyroid Hormone and Hematological Indices Among Lead Exposed Jewelry Workers in Dhaka, Bangladesh

I. Mazumdar¹ · K. Goswami² · Md Suhrab Ali³

Received: 15 January 2016 / Accepted: 17 May 2016 / Published online: 25 May 2016
© Association of Clinical Biochemists of India 2016

Abstract Jewelry utilizes lead either directly or as a base metal. Costume jewelry requires lead before molding and plating the product with valuable metals. Therefore, such ornaments have a great potential to release heavy metals having health hazards. Also, jewelry makers engaged in preparing German silver, an alloy, apply lead in smelting, alloying, rolling and milling silver wires and pieces. The metal is taken up by blood, soft tissues and bone. The biological effects of lead are dependent upon the level and duration of exposure. Lead inhibits three enzymes of heme biosynthesis- δ -amino-levulinic-acid dehydratase (ALAD), coproporphyrin oxidase, and ferrochelatase, impairing heme synthesis and depressing serum level of erythropoietin resulting in decreased hemoglobin synthesis. Lead exposure also affects calcium metabolism and impair the synthesis of Calcitriol. In the present study, jewelry makers from Dhaka, Bangladesh, were shown to have significantly high levels of lead, protein, albumin, and parathormone in their blood, and significantly high amount of zinc-protoporphyrin and δ -amino-levulinic-acid in their urine. The control group, on the other hand showed significantly higher amounts of calcium (both total and ionized form) Vitamin D₃ and non-activated erythrocyte ALAD in their blood, along with hemoglobin. It might be due to inhibition

of 1- α -hydroxylase enzyme in renal tubules. Lead causes nephro-toxicity and inhibits 1- α -hydroxylase enzyme leading to decreased calcitriol synthesis resulting in impaired calcium absorption across gastro-intestinal tract and renal tubules. Low Vitamin D₃ and significantly increased Parathyroid hormone (PTH) in study group has been found.

Keywords Calcium · Vitamin D · Parathyroid hormone · Lead

Introduction

Jewelry is viewed as a form of personal decoration or adornment. The processing of jewel manufacturing utilizes lead either directly or as a base metal for other precious objects. The products include valuable metals like gold, silver and platinum and what is known as ‘costume jewelry’. In this, lead is mixed before molding and plating the product with valuable metals [1]. Therefore, such ornaments have a great potential to release heavy metals that have health hazards. Further, it has been reported that the high amount of lead that can be present in such ornaments, had a serious consequence involving the death of a 4 year old child in USA, due to swallowing of a piece of costume jewelry containing above 99 % lead. [2]. Also, jewelry makers engaged in preparing of German Silver, an alloy, apply lead in smelting, alloying, rolling and milling silver wires and pieces. They have also been found to have high blood lead levels [3].

Lead, a ubiquitous and versatile metal that has been used by mankind for over 6000 years is today one of the most widely distributed toxins in the environment. It is present in food, beverages, soil/dust particles, and atmosphere, and

✉ I. Mazumdar
mazumdaripsita@gmail.com

¹ Department of Biochemistry, KPC Medical College and Hospital, Raja S C Mullick Road, Jadavpur, Kolkata, West Bengal 700 032, India
² Department of Biochemistry, Lincoln University College, Kuala Lumpur, Malaysia
³ Department of Biochemistry, Bangabandhu Sheikh Mujib Medical University, Dhaka, Bangladesh

is absorbed in the gastro-intestinal tract. The metal is rapidly taken up by blood and soft tissues (half life-28–36 days) and then bone (half life-27 years).

The biological effects of lead are dependent upon the level and duration of exposure. Lead inhibits 3 enzymes of heme biosynthesis- δ amino levulinic acid dehydratase (ALAD), coproporphyrin oxidase, and ferrochelatase. This effects erythrocyte formation by impairing heme synthesis and depresses serum level of erythropoietin, a hormone that regulates erythrocyte formation [4].

Lead exposure also affects calcium metabolism in various ways. Toxic lead levels can impair the synthesis of the most important Vitamin D metabolite, dihydroxy Vitamin D (Calcitriol), by inhibiting renal absorption [5]. Till date only one published study has examined the relationship between Vitamin D deficiency and anemia, and has found positive association between the two in adults [6].

Current knowledge on the overall effect of occupational lead exposure on the parathyroid hormone-Vitamin D-calcium axis remains unclear.

The present study included the biological monitoring in workers exposed to lead from the jewelry manufacturing industry and includes estimation of blood lead levels, urine ALAD and blood hemoglobin levels. In addition, serum calcium, Vitamin D and parathyroid hormones were estimated to study the effect produced by lead levels on them. Further, attempts were made to study association between blood lead levels and other parameters as surrogate biomarkers for lead exposure.

Materials and Method

The present study was conducted on the workers and staffs of jewelry workshop in Dhaka, Bangladesh, who agreed to participate in the study, in the month of February, 2015.

Study Sample

Forty-seven subjects (sample size) were selected, who were employed full time (average 8–12 h/day), for the last 10–25 years and exposed to the fumes and dusts of lead. A control group of 42 people, who were background office staffs, not directly exposed to toxic fumes but working in the vicinity of the production units, were selected, whose age distribution and environmental condition were standardized to those of the subject group. Hence the total sample size was 89.

Exclusion Criteria

Chronic diseases that affect Vitamin D status such as malabsorption, chronic liver disease, renal impairment,

thyroid dysfunction, supplementation, medications causing Vitamin D deficiency like Rifampicin, Ketoconazole, Phenytoin, Valproate, Corticosteroids, Orlistat etc., and a family history of Vitamin D deficiency were all excluded from the study.

Pilot Study

A pilot study was done before starting on 20 % of the sample size (10 persons) to test the feasibility of the study parameters in enquiring about the concerned risk factors. Those included in pilot study were not included in the main study results to avoid contamination of samples.

Ethical Issues

Before interview, workers and staffs were informed about the general aim of the study and their participation in the study was absolutely voluntary. Confidentiality of collected data was guaranteed to participants and informed consent was obtained. The entire experimental protocol was approved by the institutional ethical committee (ethical committee of Bangabandhu Sheikh Mujib University) and utmost care was taken during the investigation to proceed according to the ethical committee declaration of Helsinki [7].

All subjects were studied within the same time period (February 2015) to avoid seasonal variation in the level of serum Vitamin D and were subjected to the following-

- Pre constructed questionnaire enquiring about socio-demographic data. The current physical activity was calculated using Global Physical Activity Questionnaire developed by WHO, based on The Metabolic Equivalent of Task (MET) values [8]. Data related to sun exposure, sunscreen use, clothing and occupational history, mode of travel to workplace, number of hours spent in workplace, and daily consumption of dairy products were obtained. Symptoms attributable to hypo Vitaminosis D like frequent fractures, bone ache, back pain, muscle spasm, muscle weakness and waddling gait were checked.
- A full clinical examination and anthropometric measurements were done for all subjects. Height and weight were measured between 6 and 9 am, without shoes, with the subject wearing only light clothes. Body weight was measured with the electronic scale; accurate to 100 gm. Quetlet's index [$\text{wt (kg)/height (m}^2\text{)}$] was used as a measure of body mass index (BMI).
- Field tests included an examination of the working conditions by an expert hygienist and monitoring of environmental lead levels.
- Venous blood samples were taken in a climate controlled room before the beginning of a regular

workday after a 10 h fast (during which subjects were encouraged to drink water), with the subject seated. The tourniquet was released immediately after blood began to enter the tube to avoid venostasis. The samples were placed in vacuum test tubes without additives, with K2 EDTA (ethylene diamine tetra acetic acid) and Sodium Heparin. Serum was separated from whole blood in the tubes without additives within 30 min of being drawn and the tube was covered with tin foil and stored at -20°C . Blood with EDTA was stored at 4°C until tests were done.

- At the time of blood collection, random urine samples were collected in a plastic bottle from each subject. After that samples were kept frozen in deep refrigerator at -70°C until time of analysis.

Heparinised whole blood and 24 h urine samples were analysed for lead levels by atomic absorption spectrophotometry (Perkin Elmer, 2380). Principle applied was formation of lead complex with 2 % ammonium pyrrolidine dithiocarbamate and its extraction into methyl isobutyl ketone. Eluted organic phase were measured at 283.3 nm wavelength with background correction and calibration by standard addition [9]. Special precautions were taken to avoid contamination by ambient lead, reagents and materials (heparinised vacutainers and urine containers). The sensitivity of the instrument is in the range of 0.1–15 $\mu\text{g/dL}$ with good accuracy and precision. All tests were done using inter laboratory quality control materials of Centre for Disease Control and Prevention (CDC), USA.

Hemoglobin was determined by Cyanohemoglobin Method [10].

Erythrocyte D amino levulinic acid dehydratase (ALAD) was estimated by the Julian Chrisohan Method [11]. 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 the following formula:

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

where, 2 = conversion factor for ALA to POG, 35 = Dilution factor, 60 = Incubation time in minutes, 0.062 = micromolar absorptivity of modified Ehrlich's Reagent and PGB chromogen.

Erythrocyte ALAD activated by Zinc acetate and ratio of activated/non activated ALAD was determined.

δ aminolevulinic acid was estimated in urine samples by the method of Wada et al. [12]. δ amino levulinic acid reacts with acetyl acetone and forms pyrrole substance, which reacts with p-dimethyl amino benzaldehyde. The colored component was measured spectrophotometrically at 555 nm. The results were expressed as mg/dL. Porphobilinogen in urine was estimated according to Manzerral and Granick's method [13]. 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 6 min. The values were calculated according to the Rimington formula [14]

$$\text{Urinary PBG (mg/dL)} = \frac{\text{optical density} \times \text{no of times the urine sample is diluted}}{70.85}$$

Total Vitamin D level was determined in serum by electrochemiluminescence immunometric assay using Roche analysis kit. According to Endocrine Society Clinical Practice Guidelines Vitamin D deficiency was defined as <20 ng/mL, insufficiency between 21 and 29 and >30 ng/mL as the optimal level [15].

Intact parathyroid hormone (PTH) was measured by a solid phase 2-site electrochemiluminescence immunometric assay with Roche diagnostic. The biological reference interval for intact PTH is 15–65 pg/mL.

Total serum calcium level was estimated using Arsenazo III, serum protein level with Biuret and serum albumin level was measured with Bromocresol Green method using the kits supplied by Roche.

Ionized calcium is calculated as follows:

$$\text{Total Calcium} = [8 \times \text{Albumin (gm/100 mL)} + 2 \times \text{Globulin (gm/100 mL)} + 3]$$

Both internal and external quality control was used, using quality control sera of BioRad, USA, with satisfactory results. The analytes Vitamin D, PTH, Calcium, Total Protein, and Albumin were measured using Roche Hitachi Cobas 6000 autoanalyser.

None of the methodologies used for the analyses in present study have been modified or altered from the original methods of estimation of those.

Statistical Analysis

Data analyses were carried out using SAS software [16]. Student's 't' test was used to compare different parameters of jewelry workers. Results were considered significant when probability (p value) was ≤ 0.05 .

Results

The characteristics of the study groups are shown in Table 1. The test and control subjects were similar in age, years of experience and body mass index. Variables in Table 1 are considered possible cofounders and are included in multivariate analysis. There were no ethnic differences between the groups (data not shown).

Table 2 summarises the lead concentration in blood and urine, hemoglobin, and other related parameters like ALAD (activated and inactivated), erythrocyte ZPP, urinary ALAD and porphobilinogen.

Table 3 measures the biochemical parameters in the jewelry workers vis-à-vis the control group. It includes total protein, albumin, calcium (total and ionized), PTH, and 25OH Vitamin D.

The hemoglobin level of the jewelry workers were found to be highly significantly decreased as compared to the control group ($p < 0.0001$). Further investigations on blood and urine regarding lead and related parameters yielded the following results.

The blood lead level was significantly increased in the jewelry workers compared to the control group ($p < 0.0001$), so was urinary lead level. The Zinc activated protoporphyrin (ZPP) level was significantly increased in the cases than controls ($p < 0.005$). Urinary δ amino levulinic acid dehydratase was very significantly increased in the workers ($p < 0.0001$), whereas the PBG level was not statistically altered ($p < 0.04$).

Non activated erythrocyte ALAD activity was found to be highly elevated in the control population as compared to the jewelry workers ($p < 0.0001$), whereas not much difference

Table 1 Demographic details of jewelry workers exposed to lead along with the control group

Parameter	Control group (n = 42)	Jewelry workers (n = 47)
Age (years)	32–62	29–65
Experience (years)	10–25	10–25
Duration of exposure (years)	10–25 (indirectly exposed to work environment with high lead level)	10–25 (directly exposed to toxic lead fumes and alloys)
BMI (kg/m ²)	22.5 ± 0.5	23.5 ± 0.5

Values represent mean ± SD

Table 2 Concentration of hemoglobin, lead and related parameters in study group and control group

Parameter	Control (n = 42)	Jewelry workers (n = 47)	p value
Hemoglobin (gm/dl)	14.7 ± 1.9	10.2 ± 1.1	<0.0001
Lead in blood (µg/dl)	16 ± 3	69 ± 13	<0.0001
Lead in urine (µg/dl)	8.21 ± 3.19	13.12 ± 7.16	<0.0001
ZPP (µg/dl)	7.26 ± 2.81	10.55 ± 2.79	0.0031
dALA in urine (mg/L)	9.76 ± 6.45	26.19 ± 10.32	<0.0001
PBG in urine (mg/L)	11.12 ± 3.14	13.06 ± 5.21	0.039
<i>Erythrocyte δ amino levulinic acid dehydratase (μmol ALA utilized/min/l of RBC)</i>			
Activated	28.18 ± 15.91	26.43 ± 7.25	0.49
Non activated	25.12 ± 4.81	20.19 ± 6.12	<0.0001
Activated : Non activated	1.12 ± 0.98	1.29 ± 1.19	0.46

Values represent mean ± SD

Table 3 Biochemical parameters in blood of lead exposed jewelry workers and controls

Parameters	Control (n = 42)	Jewelry workers (n = 47)	p value
Total protein (gm/dl)	7.2 ± 0.12	6.3 ± 0.2	<0.0001
Albumin (gm/dl)	4.3 ± 0.16	3.3 ± 0.15	<0.0001
Total calcium (mg/dL)	9.8 ± 1.8	7.7 ± 2.6	<0.0001
Ionized calcium (mg/dL)	5.5 ± 0.9	4.2 ± 1.2	<0.0001
PTH (pg/mL)	52.9 ± 7.8	167.6 ± 21.2	<0.0001
25 OH Vitamin D (ng/mL)	32.6 ± 9.6	12.8 ± 3.9	<0.0001

Values represent mean ± SD

was observed between values of activated erythrocyte δ amino levulinic acid dehydratase activity ($p = 0.49$). The activated: non activated erythrocyte δ amino levulinic acid dehydratase ratio (i.e. $\mu\text{mol ALA utilized}/\text{min}/\text{L}$ of RBC) between the study group and the control group was not statistically significant either ($p = 0.46$).

Regarding the biochemical parameters, the following results were observed.

In the jewelry workers, serum total protein and albumin levels were found to be significantly reduced as compared to the office staff ($p < 0.0001$). The concentration of both total and ionized calcium were seen to be significantly raised in the control group as compared to the study group ($p < 0.0001$). A strong negative co-relation was observed between the active vitamin D_3 level and PTH level; while the 25OH-Vitamin D_3 level was significantly high in the office staffs as compared to the study group ($p < 0.0001$), the PTH level was found to be significantly lower in them as compared to the jewelry workers ($p < 0.0001$).

Discussion

The study examined the effect of occupational lead exposure on calcitrophic hormones, blood calcium-Vitamin D homeostasis, hemoglobin and lead affected products in blood and urinary D-ALA changes among jewelry workers. In the present study, we observed that there is significant elevation of blood lead level and urinary lead level in jewelry workers as compared to the controls, who, incidentally, also have a slightly higher blood lead value than the permissible limit of $10 \mu\text{g}/\text{dL}$ for the general population, due to the indirect exposure. Generally lead absorption results in rapid urinary excretion, but if excessive exposure continues, the metal accumulates in bone. When bone storage capacities are exceeded, it moves into soft tissues. The blood lead level depends on the equilibrium between absorption, storage and excretion. It generally reflects acute or current exposure because of the short 1/2 life of lead in blood (28–36 h), but it is also influenced by previous storage [4]. Blood lead level is the best and most sensitive biomarker for identifying lead pollution, current exposure and adverse effects. Also, the urine lead excretion has been considered as an index of exposure; since blood lead values change more rapidly than urine excretion, but estimation of urinary lead seems to be of limited use for general screening, because of various influences by renal function, fluid intake and specific gravity of urine [17].

Condensation of two molecules of δ ALA to form the monopyrrole PBG is catalyzed by δ amino levulinic acid dehydratase (E.C. 4.2.1.2.4). The δ -ALA is a zinc dependent metallo enzyme and zinc partly protects this enzyme against the adverse effect of lead in vitro and possibly also in vivo [18].

The RBC-Zinc Protoporphyrin (ZPP) concentration was highly altered in the jewelry industry workers as compared to the control group. The ZPP level was elevated in about 50–75 % of those who have the range of blood lead levels 40–60 $\mu\text{g}/\text{dL}$ without any symptoms and are almost always elevated in symptomatic lead poisoning [4].

Decrease of hemoglobin synthesis and anemia as an effect of lead on the hematopoietic system is observed in children and adults [4]. 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 regulates erythrocyte formation, and also decreases erythrocyte survival through its inhibition of membrane bound $\text{Na} + \text{K} + \text{ATP-ase}$ [4]. In the present study the hemoglobin levels were found to be significantly higher in the control group ($p < 0.0001$) than the study group, highlighting the preponderance of anemia in the jewelry workers.

Serum total calcium levels were significantly decreased ($p < 0.0001$), and serum ionized calcium levels were also significantly decreased in jewelry workers as compared to controls. Serum Vitamin D levels were decreased ($p < 0.0001$) in workers as compared to office staff, whereas parathyroid hormone levels were significantly elevated ($p < 0.0001$). Decreased serum total calcium and ionized calcium in jewelry makers maybe due to increased blood lead because lead inhibits 1α -hydroxylase enzyme in renal tubule, which is required for calcitriol formation. Calcitriol plays a crucial role to maintain homeostasis of calcium metabolism. It stimulates the synthesis of calcium binding protein (CBP) in intestine, which is required for absorption of calcium across small intestine. It also facilitates absorption of calcium at renal tubules. In this study increased blood lead may decrease Calcitriol (1, 25, DHCC) concentration by similar mechanism, resulting in hypocalcemia [19].

This decreased calcium levels confirms earlier experimental and clinical reports and reflects disturbance of calcium metabolism due to lead [20]. Lead is a biochemical analogue of calcium, thus it interferes with calcium in several metabolic pathways. One study also shows the interference of lead with the final metabolism of Vitamin D to the active metabolite Calcitriol (1, 25, DHCC), a hormone required for adequate calcium absorption [5].

Vitamin D, the sunshine Vitamin has received a lot of attention recently as a result of a meteoric rise in the number of publications showing that it plays a crucial role in a plethora of physiological functions [21]. Background studies of Vitamin D deficiency and insufficiency are prevalent worldwide, but relatively few studies have examined Vitamin D status in working populations [22]. About Vitamin D level in males, specially who work mainly indoors and who are presumably at risk of Vitamin

D deficiency; it should be noted that only 10 % of total requirement of Vitamin D comes from diet. Thus, the major cause of Vitamin D deficiency seems to be inadequate exposure to sunlight [15].

The mechanism of production of ionized calcium is still not clearly understood. It can be speculated that parathyroid hormone (PTH) which has a more direct effect on level of ionized calcium is also perturbed by elevated lead levels [23]. It indicates that lead is an endocrine modulator and can thus be responsible for endocrinal disturbances. We measured serum Vitamin D in jewelry workers, whose serum calcium levels were drastically low and parathyroid levels high due to constant lead exposure. Estela Kristal-Bouneh reported that the levels of both Vitamin D and PTH were significantly increased in the lead exposed workers which helped in maintaining the normal serum calcium levels in those workers. Our results are contrary to the report. Theoretically at high blood lead level calcitriol concentration decreases due to inhibition of 1- α -hydroxylase enzyme in renal tubule resulting in hypocalcemia which further stimulates secretion of PTH.

The present findings of serum concentration of calculated ionized calcium in the 2 groups probably reflects the tight regulation of this mineral and suggests that the elevated PTH level among lead exposed subjects is likely to be the contradictory outcome of a tendency to systemic hypocalcemia. In addition, it is known that PTH synthesis is also stimulated by β adrenergic agonists and though we cannot rule out such a mechanism, it has been pointed out that this is a minor effect physiologically compared the effect produced by ionized calcium [23].

The serum protein and albumin levels were significantly lower in the exposed group.

In conclusion, the study clearly indicates that serum total calcium, ionized calcium, and Vitamin D levels are significantly decreased and PTH level is significantly increased in jewelry workers as compared to control groups. It may be due to inhibition of 1- α -hydroxylase enzyme in renal tubules. Lead causes nephrotoxicity and inhibits 1- α -hydroxylase enzyme which leads to decreased calcitriol synthesis resulting in impaired calcium absorption across GIT and renal tubules. Significantly increased PTH in study group might be due to hypocalcemia.

These parameters, if measured regularly in people exposed to similar kind of Lead toxicity, might act as surrogate biomarkers for the duration and level of lead exposure.

Limitations of the Study

The obvious limitation of the present study is the cross sectional nature. Also, it cannot measure direct causation between indoor jewelry workers and Vitamin D deficiency.

Secondly the sample of the study was obtained from one geographical area. So, geographic and habitual limitations should be taken into consideration before any attempt of generalization is made.

Acknowledgments The authors are grateful to the National Referral Centre for Lead Projects in India, West Bengal, (NRCLPI.WB), for providing laboratory facilities and providing financial support.

Funding The study was financially supported by the National Referral Centre for Lead Project in India, West Bengal, (NRCLPI,WB) (award number-006/13-13). Recipient- Prof. K. Goswami.

Compliance with Ethical Standards

Conflict of interests Dr I. Mazumdar, Prof K. Goswami and Prof Md Suhrah Ali have declared that no conflict of interest.

Ethical Approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed Consent Informed consent was obtained from all individual participants included in the study.

References

- Olivier P, Wallace J. Digital technologies and the emotional family. *Int J Hum Comput Stud.* 2009;67:204–14.
- Berg KK. Death of a child after ingestion of a metallic charm. *CDC* 2006;55(12):340–51.
- Goswami K, Banerjee SK, Shuhrah S, Bhattacharya B. Ascorbic acid therapy in lead exposed jewelry workers of Bangladesh. *Showa Univ J Med Sci.* 2001;13(1):11–6.
- WHO. Biological indices of lead exposure and body burden. *IPCS Inorg Lead Environ Health Criteria.* 1995;118:114–8.
- Sharma P, Purohit P. Lead exposure exacerbates cardiovascular risk. *Indian J Clin Biochem.* 2014;29(2):117–8.
- Sim JJ, Lac PT, Liu IL. Vitamin D deficiency and anemia: a cross sectional study. *Ann Hematol.* 2009;89:447–52.
- Declaration of Helsinki. Amended by World Medical Assembly, Venice, Italy, 1964 & 1983. *BMJ* 1996; 3/3(70): 1448–9.
- World Health Organization Chronic diseases and Health Promotion: Global Physical Activity Surveillance. 2005 (cited March 2013). <http://www.who.int/ehp/steps/GPAQ/en/index.html>.
- Zinterhofer LJM, Jatlow PI, Fappiano A. Atomic absorption determination of lead in blood and urine in the presence of EDTA. *J Lab Clin Med.* 1971;78:664–74.
- Drabkin DL, Austin JH. Spectrophotometric studies: spectrophotometric constant for common hemoglobin derivatives in human, dog and rabbit blood. *J Biol Chem.* 1932;98:719–33.
- Chisholm JJ Jr, Thomas DJ, Hamill TG. Estimation of δ -ALA activity from erythrocytes. *Clin Chem.* 1986;94(3):601–5.
- Wada O, Wada S, Kohei T, Gumpei U, Yuza Y, Kiku N, et al. A simple method for the quantitative analysis of urinary δ -ALA to evaluate lead absorption. *Br J Ind Med.* 1969;26:240–3.
- Manzeral D, Granick S. The occurrence and determination of δ -ALA and PBG in urine. *J Biol Chem.* 1956;219:435–46.
- Rimington A, Sears WG, Eales L. Symptomatic porphyria in a case of Felty's syndrome II. Biochemical investigations. *Clin Chem.* 1971;18:462–70.

15. Holik MF, Binkley NC, Bischoff-Ferrani HA, Gordori CM, Haley DA, Heaney RP, et al. Evaluation, treatment and prevention of vitamin D deficiency; an endocrine society guideline. *J Clin Endocrinol Metab.* 2011;26(1):1911–30.
16. SAS Institute Inc, SAS version 6.12, Cary: SAS Institute, Inc. 1996.
17. Ellis RW. Urinary screening tests to detect excessive lead absorption. *Br J Int Med.* 1966;23(2):263–75.
18. Tsukamoto I, Yoshinagu T, Suno S. The role of Zinc with special reference to the essential thio groups in d-ALAD of bovine liver. *Biochem Biophys Acta.* 1979;570:165–78.
19. Kristal Bouneh E, Froom P, Yerushalmi N, Harari G, Ribak J, Sakari M, et al. Calcitropic hormones and occupational lead exposure. *Am J Epidemiol.* 1998;147(5):458–63.
20. Anetor JI, Akingbola TS, Adeniyi FA, Taylor GO. Decreased total and ionized Calcium levels and Hematological indices in occupational lead exposure as evidence of the endocrine disruptive effect of lead. *Int J Occup Environ Med.* 2005;9(1):15–21.
21. Wacker M, Holick MF. Vitamin D-effects on skeletal and extraskeletal health and the need for supplementation. *Nutrients.* 2013;5(1):111–48.
22. Norris JM. Can the sunshine vitamin shed light on type 1 diabetes? *Lancet.* 2001;358:1476–8.
23. Kukreja SC, Hargis GK, Bowser EN. Role of adrenergic stimuli in parathyroid secretion in man. *J Clin Endocrinol Metab.* 1978;40:478–81.