Original Article

Blood lead levels among the occupationally exposed workers and its effect on calcium and vitamin D metabolism: A case-control study

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Abstract

Introduction: Lead (Pb) is one of the major occupational pollutants present in the developed and developing countries including India. In humans, Pb can cause a wide range of biological effects depending upon the level and duration of exposure. The goal of this study was to evaluate the blood lead levels (BLLs) and its associated effects on vitamin D and calcium metabolism, among the workers occupationally exposed to Pb. **Materials and Methods:** This cross-sectional, case-control study was conducted for a period of 18 months (January 2017 to July 2018). A total of 160 subjects were included in the study (80 in each, Pb-exposed group and control group). The blood Pb levels were quantified by using an inductively coupled plasma mass spectrometry with triple quadrupole technology (iCAP™ TQ ICP-MS). Other biochemical parameters were estimated using fully automatic analyzer by RANDOX, RX-imola, Crumlin, UK and Johnson and Johnson, VITROS® EC*i*Q, Immunodiagnostic system, Ortho Clinical Diagnostics, New Jersey, USA. **Results:** Upon analysis it was observed that serum calcium, phosphorous, and vitamin D levels were significantly decreased (8.35 ± 0.42 mg/dl, 3.07 ± 0.34 mg/dl, and 28.82 ± 10.81 ng/ml respectively; $P < 0.001$), whereas the BLL and serum iPTH levels were significantly increased (38.02 ± 19.92 µg/dl and 116.78 ± 19.93 pg/ml respectively; $P < 0.001$) in Pb exposed subjects as compared to control subjects. **Conclusion:** Our study results demonstrated that high BLL significantly alter vitamin D and calcium metabolism. The data extrapolated from our study emphasizes the necessity of surveillance in exposed workers. As the associated deleterious effects of Pb-exposure can be serious, we propose that a routine-periodical screening of the workers exposed to lead should be conducted.

Keywords: Blood lead level, calcitriol, hypocalcemia, hypophosphatemia, nephrotoxicity, parathyroid hormone

Introduction

Lead (Pb), one of the most widely distributed toxic metals in the environment, has been used by mankind for over 9000 years. Pb demonstrates some unique physical and chemical properties like malleability, ductility, corrosion resistance, poor conductivity, softness, high thermal expansion, low melting point, and easy workability, which lead to its widespread use in various industries including battery manufacturing, smelting, jewelry making, mining, paints, ceramics, porcelain, and folk remedies.[1,2] However, it has been associated with several adverse health effects and, hence, currently has become a global health concern.[3]

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As such, there is no known human physiological value of Pb, and there is no safe level of exposure to this xenobiotic. Previously as per the Center for Disease Control (CDC, 2011) reference, blood lead levels (BLL) of <10 μg/dl in adults were considered acceptable;[4,5] however, it is now known that even low BLL (<10 μg/dl) are believed to be associated with abnormalities in hematopoietic, nervous, and renal systems.[6] Consequently in year 2015, The National Institute of Occupational Safety and Health (NIOSH)/CDC designated 5 μg/dl of Pb, in a venous blood sample, as the reference BLL for adults.[7]

Environmental and occupational exposures are the two major modes that contribute to Pb toxicity through ingestion, inhalation, and dermal contact.[4] On exposure, Pb is readily absorbed by the gastrointestinal tract and is rapidly taken up by the blood and soft tissues (half-life: 28–36 days) followed by a slower redistribution to bones (half-life: 27–30 years). Pb mainly accumulates in RBCs, soft tissues (brain, kidney, and bone marrow), and mineralized tissues (bones and teeth). Excretion from body is mainly through urine (>90%) and partly through sweat, hairs, feces, and nails.[1,2]

Despite stringent regulations and policies, Pb is still one of the major occupational pollutants present in the developed and developing countries including India. Depending upon the level and duration of exposure, Pb can cause a wide range of biological effects in humans.[8] Due to the widespread use of Pb in various industrial enterprises, occupational exposure to Pb among the workers can cause significant toxic effects in the central nervous, hematopoietic, renal, gastrointestinal, cardiovascular, and reproductive systems of their body.[9] Moreover, studies have demonstrated that long-term Pb exposure may contribute to an increased risk of cancer development.[10] The International Agency for Research on Cancer has classified inorganic Pb compounds into 2A group of probable human carcinogens.[8]

There are many proposed mechanisms of toxic lead action. It has been established that Pb induces oxidative stress by the generation of reactive oxygen species (ROS) and impairment of antioxidant defenses. Pb predominantly inhibits the enzymes of heme biosynthesis and depresses serum erythropoietin level, thereby impairing the effective erythrocyte formation.[10] In addition, Pb affects mineral metabolism mainly calcium and phosphorous by inhibiting 1- α-hydroxylase enzyme in renal tubules which is required for the synthesis of most important vitamin D metabolite, dihydroxy vitamin D (calcitriol), this, in turn, minimizes calcium and phosphorous absorption from the intestines and renal tubules, leading to hypocalcemia and hypophosphatemia.[11,12] Vitamin D plays a key role in bone metabolism and studies suggest that BLLs affect the synthesis of 1, 25-dihydroxy vitamin D in kidney.[13] Taken together, these effects decrease bone mineralization, thereby decreasing bone mineral density, and increase the risk of osteoporosis particularly in occupational Pb exposed population.

Pb plays an important role in several industries in India; hence, biomonitoring of occupationally exposed subjects and identifying the associated deleterious effects becomes essential. The goal of this study was to evaluate the BLLs and its associated effects on vitamin D and calcium metabolism, among the workers occupationally exposed to Pb.

Materials and Methods

Study duration

This cross-sectional, case-control study was conducted in Department of Biochemistry, Santosh Medical College, Ghaziabad and Jaipur National University Institute of Medical Sciences and Research Centre, Jaipur for a period of 18 months (January 2017 to July 2018. Prior to the sample collection, approval from the Institutional Ethical Committee (IEC) was obtained. IEC certificate no. from both the institute's is as under: (SU/2015/793(10), dated 17.06.2015 and JNUIMSRC/IEC/2016/24, dated 05.01.2016).

Study population

The study protocol and the objectives of the study were explained to the enrolled subjects and their written informed consent was obtained. The selection of the subjects was based on a predesigned questionnaire including demographic details, medical history (about medications, vaccinations, prior illness if any, and exposure to X-rays), lifestyles (smoking, chewing tobacco, and alcohol intake), and occupational exposure to Pb (working hours/day, years of exposure, use of protective gear, etc.). A total of 160 subjects were included in the study (80 in each, Pb-exposed group and control group).

Pb-exposed group

The subjects included in this group were building construction workers including the ones involved in tiles and granite cutting, painters, motor garage workers, denting and painting workers, and battery workers involved in removing Pb electrodes, smelting, recycling of Pb batteries, and manufacturing and assembling Pb-acid storage batteries. The age of Pb-exposed subjects ranged from 15 to 47 years and they were exposed to Pb from 3 to 12 years, with a daily exposure ranging from 4 to 10 h.

Control group

The subjects included in this group were also selected based on the questionnaire and belonged to general population with no history of occupational exposure to Pb or any known physical or chemical agent in the workplace but belonged to the same age group and socioeconomic status as that of the Pb-exposed group.

The subjects who smoked >5 cigarettes or bidi/day or chewed tobacco at least 5 times/day and who took 5 glasses of an alcoholic drink/day for at least past 12 months were considered as smokers and alcoholics, respectively, in both Pb-exposed and control groups. The subjects, who were suffering from diabetes mellitus, hypertension, had a prior history of any major illness or had undergone any surgery in the recent past, were excluded

from the study. In addition, the subjects who gave a history of chronic disease affecting vitamin D status (chronic liver disease, thyroid dysfunction, renal disease, malabsorption, etc.), or being on medications causing vitamin D deficiency (corticosteroids, valproate, phenytoin, rifampicin, ketoconazole, etc.) were also excluded from the study.

Sample collection

Blood samples were collected from the Pb-exposed subjects and control subjects and were coded to avoid possible bias. A total 10 ml of venous blood was collected from each subject by using sterilized syringes. From 10 ml, a total of 5 ml blood was dispensed into EDTA vacuum tube for the estimation of BLL. The remaining 5 ml was dispensed in a plain vial without anticoagulant to extract serum after centrifugation at 2,500 rpm for 10 min. The extracted serum was used for the estimation of biochemical parameters including serum calcium, phosphorous, 25OH-Vitamin D (Vitamin D), and intact parathyroid hormone (iPTH). The samples were transported to a laboratory in a leak-proof box with cool packs and were processed within 2 h after collection.

Blood lead estimation

The BLL was quantified by using an inductively coupled plasma mass spectrometry with triple quadrupole technology (iCAP™ TQ ICP-MS, Thermo Fisher Scientific, Bremen, Germany). The EDTA blood was homogenized for 10 min by mechanical shaking; thereafter blood plasma (1.0 ml) was gravimetrically diluted (in precleaned polypropylene bottles kept in 2% nitric acid for 72 h) with 0.5% m/m nitric acid (Fisher Scientific) and 2% m/m tetramethylammonium hydroxide (Merck, Sigma Aldrich) in ultrapure water. A calibration blank, a series of standards, and a quality control were prepared by using the same procedure. All samples and standards were spiked with an internal standard mix (10 µg/L of ^{209}Bi). The sample digests were filtered with Wattman paper several times to obtain a clear solution. The diluted digests were measured directly by ICP-MS, and the concentration of Pb in blood was quantified as µg/dl.

Biochemical parameters

The biochemical parameters like serum AST, ALT, ALP, LDH, total protein, albumin, urea, creatinine, calcium, and phosphorus were estimated using fully automatic analyzer by RANDOX, RX-imola, fully automatic analyzer, Crumlin, UK. Serum vitamin D and iPTH levels were estimated by enhanced chemiluminescence technique using fully automatic analyzer by Johnson and Johnson, VITROS® ECiQ, Immunodiagnostic system, Ortho Clinical Diagnostics, New Jersey, USA.

Statistical analysis

The data were analyzed using Statistical Package for Social Sciences (SPSS 21.0). The significance of the differences between controls and exposed subjects' endpoint means was analyzed using Student's *t*-test. Results were considered significant when probability (*P*-value) was <0.001. Values were expressed as mean ± standard deviation (SD).

Results

The demographic details of the study subjects of both the groups were summarized by age, years of exposure, daily exposure, smoking/tobacco chewing habit, and alcohol consumption and are depicted in Table 1. The mean age of the Pb-exposed subjects was 30.90 ± 5.75 years and of control group subjects was 30.24 ± 6.01 years and was almost similar. The mean duration of exposure in years and daily exposure in hours for Pb exposed study subjects was 8.57 ± 3.81 years and 5.82 ± 1.69 h, respectively. The other general parameters obtained were smoking/chewing tobacco and alcohol consumption [Table 1].

Table 2 summarizes the comparison of BLL, serum calcium, phosphorous, iPTH, and vitamin D (25OH-Vitamin D) levels among the Pb exposed subjects and control subjects. Upon analysis it was observed that serum calcium, phosphorous, and vitamin D levels were significantly decreased (8.35 ± 0.42 mg/dl, 3.07 ± 0.34 mg/dl, and 28.82 ± 10.81 ng/ml, respectively; $P < 0.001$), whereas the BLL and serum iPTH levels were significantly increased (38.02 ± 19.92 µg/dl and 116.78 ± 19.93 pg/ml, respectively; $P < 0.001$) in Pb exposed subjects as compared to control subjects. A strong negative

Table 1: Demographic details of the study subjects. (*n*=160)

Demographic factors	Pb exposed subjects (*n*=80)	Control subjects (*n*=80)
Age (years)	30.9±5.75	30.24±6.01
Exposure to Pb (years)	8.57±3.81	-
Daily exposure to Pb (h)	5.82±1.69	-
Smoking/Tobacco chewing		
Yes	65 (83%)	68 (85%)
No	15 (17%)	12 (15%)
Alcohol Consumption		
Yes	65 (83%)	67 (83.7%)
No	15 (17%)	13 (16.3%)
Sex Ratio		
Male	71 (88.7%)	71 (88.7%)
Female	09 (11.3%)	09 (11.3%)

Table 2: Comparison of blood lead levels, serum calcium, phosphorous, iPTH, and vitamin D levels among lead exposed subjects and control subjects

Biochemical Parameters	Pb exposed subjects (*n*=80)	Control subjects (*n*=80)
BLL (µg/dl)	38.02±19.92**	2.33±1.21
Calcium (mg/dl)	8.35±0.42**	9.33±0.47
Phosphorus (mg/dl)	3.07±0.34**	3.60±0.52
iPTH (pg/ml)	116.78±19.93**	51.91±9.57
Vitamin D (ng/ml)	28.82±10.81**	37.02±7.77

BLL: Blood lead level, iPTH: Intact parathyroid hormone. Data represented as mean±standard deviation. Significantly different from control at *P*<0.001**

correlation was observed between the vitamin D level and iPTH level; while the 25OH-Vitamin D level was significantly low among the Pb exposed subjects, the iPTH level was found to be significantly higher in them as compared to the control subjects.

Regarding the other biochemical parameters, the following results were observed.

In the Pb exposed subjects, serum AST, ALT, ALP, LDH, urea, and creatinine were significantly increased (39.69 ± 6.07 U/L, 32.75 ± 5.78 U/L, 153.83 ± 9.66 U/L, 377.09 ± 59.09 U/L, 49.54 ± 16.80 mg/dl, and 1.19 ± 0.295 mg/dl, respectively; $P < 0.001$), whereas serum total protein and albumin levels were significantly decreased (6.68 ± 0.46 g/dl and 3.51 ± 0.37 g/dl, respectively; $P < 0.001$) as compared to the control subjects. Table 3 depicts the results of routine biochemical parameters investigated.

Discussion

Pb is one of the most important occupational pollutants in today's environment and due to its unique physio-chemical properties and nonbiodegradable nature, it is accumulating in the environment at a very alarming rate. Although many developed countries have discouraged its use, still multiple industries, *viz.,* Pb based painting, battery manufacturing, recycling, refining, smelting, etc., are highly dependent on Pb. Due to significant toxicity associated with Pb, CDC[5] has defined the standard BLL to be <10 μg/dl in adults and also has emphasized on biomonitoring in occupationally exposed workers.[3,5]

In this study BLL, serum vitamin D, calcium, phosphorous, and iPTH were estimated and compared between Pb exposed subjects and control subjects. We observed a significantly high BLL in Pb exposed subjects as compared to the control subjects. Our findings were in tandem to the previous studies from different parts of India, wherein high BLLs were reported among the occupationally exposed subjects, and the values ranged from 25.26 to 65.50 μg/dl.[12,14,15] Basit *et al.*[16] and Ahmad *et al.*[17] from Pakistan, and Ahmad Akhter *et al.*[18] from Bangladesh also had similar observations and reported significantly high BLLs among the occupationally exposed workers. In our study, the mean BLL among the lead-exposed subjects was found to be 38.02 ± 19.92 μg/dl and corroborates well with the previous national and international studies. The BLL depends on the equilibrium between absorption, storage, and excretion. Over 95% of blood Pb is bound to the erythrocytes and seems to be in dynamic equilibrium with plasma Pb.[19] It has a short half-life in blood (28–36 h) and, hence, blood Pb concentrations are currently regarded as the best and most reliable biomarker for identifying Pb pollution, current exposure, and adverse effects.

Table 3: Routine biochemical parameters in the blood of lead exposed subjects and control subjects (*n*=160)

Biochemical Parameters	Pb exposed subjects (*n*=80)	Control subjects (*n*=80)
AST (U/L)	39.69±6.07**	23.11±2.99
ALT (U/L)	32.75±5.78**	19.01±2.7
ALP (U/L)	153.83±9.66**	106.29±14.44
LDH (U/L)	377.09±59.09**	256.18±15.58
Total Protein (g/dl)	6.68±0.46**	7.08±0.53
Albumin (g/dl)	3.51±0.37**	4.12±0.21
Urea (mg/dl)	49.54±16.80**	30.36±7.34
Creatinine (mg/dl)	1.19±0.295**	0.99±0.17

AST: Aspartate aminotransferase; ALT: Alanine aminotransferase; ALP: Alkaline phosphatase; LDH: Lactate dehydrogenase. Data represented as mean±standard deviation. Significantly different from control at $P<0.001$**

As the duration of Pb exposure increases, it also increases the body burden of Pb. Similarly, in our study also the duration of exposure positively impacted the BLL, and the Pb exposed subjects who had exposure to Pb for longer duration (>8 years) had higher BLL compared to the subjects who were exposed for shorter duration (<8 years). The finding was in parallel with the previous studies, wherein the prolonged duration of exposure was reported to have been associated with high BLL and consequently may have significant effects on central nervous, hematopoietic, renal, gastrointestinal, cardiovascular, and reproductive systems, bone-mineral metabolism, etc.[9,20]

We further observed that serum calcium, phosphorous, and vitamin D levels were significantly lower and the serum iPTH levels were significantly higher among the Pb exposed subjects when compared to the control subjects [Table 2]. As high BLL has been suggested to cause disruption of renal hydroxylation of 25OH-Vitamin D by 1- α hydroxylase enzyme, there is a decrease in the production of the active form of vitamin D, i.e. 1,25 $(OH)_2$ D.[21] This consequently leads to reduced calcium levels. Thus, Pb interferes with various vitamin D functions involved in calcium balance and metabolism in various tissue and organs.[22] Normally active vitamin D_3 (calcitriol) plays a crucial role to maintain homeostasis of calcium and phosphorous metabolism. It stimulates the synthesis of calcium-binding proteins in intestine, which are required for intestinal absorption of calcium. Moreover, it also facilitates absorption of calcium and phosphorous at renal tubules. Increased BLL may decrease calcitriol concentration resulting in hypocalcemia and hypophosphatemia.[23,24] Elevated BLL also cause perturbation of PTH,[25] which has more direct effect on serum calcium and in the state of hypocalcemia, PTH secretion is stimulated which leads to increased serum PTH levels. Thus, it indicates that Pb is an endocrine modulator and can possibly be responsible for endocrine disturbances. Thus, in the present study, high BLL might have led to decreased vitamin D levels which, in turn, lead to decrease in serum calcium and phosphorous levels and increase in iPTH in blood of the Pb exposed subjects.

It is also important to understand here that majority of workers occupationally exposed to Pb are economically deprived and rarely approach any tertiary care facility where the advanced diagnostic facilities are available, the reason being either the money constraints or lack of knowledge about the serious health hazards associated with prolonged exposure to Pb. Most

of these workers turn up to primary health care or community health care facilities, and a well-informed primary care physician can treat/guide such patients effectively and efficiently. The data extrapolated from our study will surely help the primary care physicians to better understand the deleterious effects associated with Pb exposure and its impact on general population as well as on workers occupationally exposed to Pb.

Conclusion

To conclude, our study results clearly indicate the significant effect of high BLLs on serum calcium, phosphorous, vitamin D, and PTH levels among the occupationally exposed workers. High BLL inhibit the 1-α hydroxylase enzyme which leads to decreased calcitriol synthesis resulting in impaired calcium and phosphorous absorption across gastrointestinal tract and renal tubules, which eventually leads to hypocalcemia and hypophosphatemia and in turn can cause a significant increase in serum PTH levels.

The data extrapolated from our study may serve as a template to tailor the guidelines regarding the necessary preventive measures to be taken in improving the working conditions and better safety measures to minimize the occupational exposure of the workers to Pb. Moreover, our study results underline the periodical screening of the workers exposed to Pb and those who are found to have BLL over and above the permissible upper limit should be briefed about the deleterious effects of the Pb exposure and the utility of timely implementation of the preventive measures to be taken.

Declaration of patient consent

The authors certify that they have obtained all appropriate participant consent forms. In the form, the participants have given their consent for their images and other clinical information to be reported in the journal. The participants understand that their names and initials will not be published and due efforts will be made to conceal their identity, but anonymity cannot be guaranteed.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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