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Assessment of Bone Turnover Biomarkers in Lead-Battery Workers with Long-Term Exposure to Lead

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Abstract

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Background: The major portion of lead in the body resides in skeletal system. The bone turnover affects the release of lead into the circulation from bones. The bone turnover biomarkers (BTM) in lead-battery workers with long-term exposure to lead have not been explored yet.

Objective: To evaluate the BTM (formation and resorption) in lead-battery workers with long-term exposure to lead in lead-battery manufacturing plant.

Methods: 176 male lead-exposed workers and 80 matched comparison group were studied. All participants were examined for blood lead levels (BLLs), bone formation biomarkers—serum osteocalcin (OC), alkaline phosphatase (ALP), bone-specific alkaline phosphatase (BALP)—and bone resorption biomarkers—serum pyridinoline (PYD), deoxypyridinoline (DPYD), tartarate-resistant acid phosphatase-5b (TRACP-5b), and urinary hydroxyproline (UHYP).

Results: We found a significantly higher bone formation biomarkers such as BALP (p=0.007) and bone resorption biomarkers, *eg*, PYD (p=0.048), TRCAP-5b (p=0.001), and UHYP (p=0.001) in lead-exposed workers. A significant (p=0.041) negative correlation (ρ -0.128) was noted between BLLs and OC. A significant positive correlation was noted between BLLs and TRACP-5b (ρ 0.176, p=0.005) and UHYP (ρ 0.258, p=0.004). Serum OC (p=0.040) and UHYP (p=0.015) levels changed significantly with BLL level. Bone resorption biomarkers levels—PYD, TRACP-5b, and BALP—were higher among those with higher BLLs levels. The duration of exposure was significantly associated with BALP (p=0.037), DPYD (p=0.016), TRACP-5b (p=0.001), and UHYP (p=0.002) levels.

Conclusion: Long-term lead exposure affects the bone turnover.

Keywords: Osteocalcin; Tartrate-resistant acid phosphatase; Alkaline phosphatase; Pyridinoline; Lead; Hydroxyproline; Deoxypyridinoline; Bone resorption

Introduction

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Lead-acid storage batteries manufacturing process involves use of hazardous chemicals of lead oxide (PbO$_2$), spongy lead, and sulfuric acid (H_2SO_4). Lead levels are expected to be high in the atmosphere of manufacturing unit.[1] Occupational exposure to lead occurs through inhalation and ingestion. Recent studies on lead-battery manufacturing workers have reported high mus-

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culoskeletal disorders with markers of inflammation,[2] mucocutaneous changes,[3] oxidative stress,[4] altered serum magnesium fractions,[5] elevated blood lead levels (BLLs),[6,7] reticulocytosis,[8] altered genetic polymorphisms of δ-aminolevulinic acid dehydratase (ALAD),[9] impaired biogenic amino acids with neurobehavioral function,[10] genotoxicity,[11] impaired coagulation function,[12] health problems,[13,14] male reproductive function,[15] ocular changes,[16] impaired hematological parameters,[17] and altered calcium metabolism by inhibiting the kidney 1-α-hydroxylase enzyme, which is required for 1, 25 dihydroxy-vitamin D_3 synthesis[18].

Bone is crucial for locomotion, body support, and organ protection.[19] Lead is deposited in cement lines of bone and associated with demineralization.[20] After long-term exposure, lead replaces the bivalent ions such as Ca^{2+}, Mg^{2+}, and Fe^2, inhibits the bone fracture healing due to delayed cartilage formation, increased resorption of collagen molecule and bone marrow oxidative stress.[21,22] A significant association was noted between BLLs and calcified cartilage turnover markers.[23] Potula, *et al*,[24] reported that lead exposure from lead-smelting process is associated with increased levels of bone resorption markers, pyridinoline (PYD) and deoxypyridinoline (DPYD), among post-menopausal women workers. High risk of bone fracture and osteoporosis was also reported in female lead-battery workers.[25] Lead-battery workers from China have reported decreased bone mineral density (BMD),[26,27] increased risk of osteoporosis,[28] increased urinary levels of hydroxyproline (UHYP), bone specific alkaline phosphatase (BALP), and osteocalcin (OC).[29] BLL was reported to be positively associated with DPYD among middle-aged lead-exposed men.[26] Levels of bone turnover biomarkers (BTM) reflect the bone formation as well as bone resorption and are responsible for

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- BLLs and bone turnover biomarkers were significantly higher among lead-exposed workers compared to the comparison group.

- Bone formation biomarker was negatively associated with BLL. Bone resorption markers were positively associated with BLL.

- Long-term lead exposure results in altered bone turnover markers.

bone remodeling status.[30] These biomarkers have been used for early diagnosis of osteoporosis.[31] The present study was conducted to evaluate the BTM of bone formation and bone resorption among workers with long-term exposure to lead in a lead-battery plant.

Materials and Methods

In a cross-sectional study, all male workers engaged in a lead-battery manufacturing plant who had been occupationally exposed to lead for at least two years were examined. The study group consisted of 176 workers working in a lead-battery plant located in Tamilnadu, India. A comparison group comprising of 80 office workers with no occupational exposure to lead were also studied. The comparison group was matched for age and socio-economic status with the study group.

Ethics Committee of the institution approved the study protocol. The study participants were informed of the study purpose and gave informed written consent to participate in the study. Demographic details, occupational history and habits of the participants (smoking and alcohol consumption) were collected by using a pre-structured questionnaire.

Sample Collection

From each participant 5 mL (2 mL in hep-

arinized tube and 3 mL in plain tubes) of whole blood and urine samples were collected. The 2-mL sample was used for estimation of BLL. The 3-mL sample was centrifuged at 3000 RPM for 10 min at 4 °C to obtain the serum for measuring BTMs. Spot urine samples were used for the measurement of UHYP and creatinine.

Blood Lead Levels

For more information on metal levels in electronic waste workers in Thailand see https://www.theijoem.com/ijoem/index.php/ijoem/article/view/1826

The 2-mL blood sample was stored at -20 °C until analysis. Using ETHOS-D, (Milestone Microwave Laboratory Systems, Italy), the sample was digested with 2 mL of nitric acid and 0.2 mL of hydrogen peroxide while maintaining power, temperature, and duration of process. The digested samples were made up to 5 mL using distilled water and centrifuged. The concentration of lead was measured using an atomic absorption spectrophotometer (GBC Avanta P, Australia). A standard solution of 20 μg/dL of lead was prepared from the stock of a standard solution obtained from the Merck and added to the lowest concentration of the sample. The analysis found 100% recovery with %RSD at <0.5 for three replicates.

Bone Formation Biomarkers

Serum alkaline phosphatase (ALP), serum BALP and OC levels were used as bone formation biomarkers. Serum ALP was measured using IFCC-AACC method.[32] Prietest clinical chemistry reagents (Robonik, India, private Ltd) were used for the estimation. In this approach, the p-nitro-phenyl phosphate is converted to p-nitrophenol and phosphate using alkaline phosphatase. The rate of formation of p-nitrophenol corresponds to ALP activity in the sample.

Quantification of BALP activity was done by using phenylalanine inhibition technique.[33] In this approach, part of serum sample was incubated with 11 mM phenylalanine solution for 20 minutes.

After incubation, residual ALP activity was measured by using the standard IFCC-AACC method.[32] The amount of BALP was calculated by subtracting the residual activity from total ALP activity.

Serum OC concentration was measured using enzyme-linked immunosorbent assay (ELISA) (YH Biosearch Laboratory, China). The absorbance of standard specimens and samples were measured using Thermo Scientific Multiskan EX-reader (USA) at 450 nm. The concentration of unknown sample was calculated using standard curve with linear regression. The range of method was 0.5–150 ng/mL; the sensitivity of the method was 0.026 ng/mL.

Bone Resorption Biomarkers

Serum pyridinoline (PYD), deoxypyridinoline (DPYD), tartarate-resistant acid phosphatase-5b (TACRP-5b), and urinary hydroxyproline (UHYP) were considered bone resorption biomarkers. Serum concentration of PYD was measured with ELISA (YH Biosearch Laboratory, China). The absorbance of standard specimens and samples were measured using Thermo Scientific Multiskan EX-reader (USA) at 450 nm. The concentration of unknown sample was calculated by using standard curve with linear regression. The assay range of method was 0.5–200 ng/mL; the sensitivity of the method was 0.024 ng/mL.

The concentration of serum DPYD was measured with ELISA (YH Biosearch Laboratory, China). The absorbance of standards and samples were measured using Thermo Scientific Multiskan EX-reader (USA) at 450 nm. The concentration of unknown sample was calculated using standard curve with linear regression. The assay range of method was 0.005–1 nmol/mL; the sensitivity of the method was 0.005 nmol/mL.

Serum TRACP-5b was measured using Sarvari, et al, method.[34] In this method,

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Table 1: Demographic characteristics of the study and comparison groups. Values are either mean (SD) or n (%).

Parameter	Study group (n=176)	Comparison group (n=80)	p value
Age (yrs)	36.6 (4.0)	37.4 (10.0)	0.361
BMI (kg/m^2)	25.7 (2.9)	25.3 (3.4)	0.339
Exposure duration (yrs)	13.3 (3.3)	0	—
Diastolic blood pressure (mm Hg)	77.7 (10.7)	74.8 (12.7)	0.059
Systolic blood pressure (mm Hg)	127.8 (14.6)	127.5 (19.1)	0.890
Alcohol consumption	95 (54%)	39 (49%)	0.437
Smoking	38 (22%)	19 (24%)	0.700
Blood lead level (µg/dL)	32 (12)	20 (6)	0.001

serum samples were diluted 10-fold with distilled water and incubated for 1 hour at 37 °C. Then, 50 µL of the diluted sample was added to 50 µL of substrate solution in a microplate. The reaction was carried out for 1 hour at 37 °C and quenched by adding 50 µL of 1 M NaOH. A calibration curve was prepared using p-nitrophenol solution of known concentrations (5−25 µg/mL in 0.05 M NaOH). The absorbance of standards and samples were measured using a Multiskan Microplate reader (Thermo Scientific) at 405 nm. The concentrations of unknown samples were determined using calibration curve with linear regression equation. One unit (1 U) of TRACP-5b activity was defined as the amount of enzyme required to hydrolyze 1 µmol of p-nitro phenyl phosphate (pNPP) per minute at 37 °C.

Quantification of UHYP was done using the modified method of Neuman and Logan.[35] In this method, hydroxyproline was treated with $CuSO_4$ and H_2O_2 in an alkaline solution with resultant formation of pyrroline-4-caroxylic acid, which is converted to pyrrole-2-caboxylic acid. This product is condensed with p-dimethyl-amino-benzaldehyde to give a red complex, which is measured in spectrophotometer at 540 nm (Elico-SL159).

Statistical Analysis

SPSS® for Windows® ver 16 was used for data analysis. *Student's t* test was used to compare the means of continuous normally distributed variables between the study and comparison group. The Spearmen's correlation test was used to examine the association between BLLs and BTM. Analysis of variance (ANOVA) was used to evaluate the effect of BLLs and duration of exposure on BTM. A p value <0.05 was considered statistically significant.

Results

Demographic details of study and comparison groups are presented in Table 1. The age, blood pressure, body mass index (BMI), and frequency distributions of alcohol consumption and smoking among study participants were matched with comparison group. The mean BLL in the study group was significantly (p=0.001) higher than that in the comparison group. The BLL among the comparison group was lesser than the World Health Organization threshold of 40 µg/dL for adults.

BALP, a bone formation biomarker, was

Table 2: Mean (SD) of bone turnover biomarkers measured in the studied groups

Biomarkers	Study group (n=176)	Comparison group (n=80)	p value
Alkaline phosphatase (U/L)	93 (28)	87 (21)	0.088
Bone-specific alkaline phosphatase (U/L)	42 (25)	34 (13)	0.007
Osteocalcin (ng/mL)	15.6 (10.0)	16.0 (5.0)	0.735
Serum pyridinoline (ng/mL)	35 (17)	31 (9)	0.048
Serum deoxypyridinoline (nmol/mL)	0.241 (0.900)	0.235 (0.400)	0.949
Serum TRACP-5b (U/L)	3.8 (2.5)	2.2 (0.7)	0.001
Urinary hydroxyproline (µg/g creatinine)	7.0 (4.5)	4.0 (3.0)	0.001

significantly (p=0.007) higher in the study group as compared with the comparison group (Table 2). The levels of bone resorption biomarkers, PYD (p=0.048), TRACP-5b (p=0.001), and UHYP (p=0.001), were also significantly higher in the study group as compared with the comparison group.

BLL had a significant negative correlation (ρ -0.128, p=0.041, Table 3) with serum level of osteocalcin and positive correlations with TRACP-5b (ρ 0.176, p=0.005) and UHYP (ρ 0.258, p=0.004).

BMTs significantly varied with BLLs (Table 4). An increasing trend of bone resorption biomarkers such as PYD and DPYD was noticed with higher levels of BLL, however this was not statistically significant. The bone formation biomarkers, *eg*, BALP, and bone resorption biomarkers, namely, DPYD, TRACP-5b, and UHYP, were significantly associated with the duration of occupational exposure to lead.

Discussion

BLL is considered a reliable indicator of recent lead exposure.[36] We found that the mean BLL in the study group was 1.6 times higher than that of the comparison group. A recent study also reports significantly higher BLLs in similar occupational

Table 3: Spearmen's correlation coefficient (ρ) between blood lead level (BLL) and bone turnover markers

Parameters	BLL	OC	ALP	BALP	PYD	DPYD	TRACP-5b
Osteocalcin (ng/mL)	-0.128*	1					
ALP (U/L)	-0.002	-0.038	1				
BALP (U/L)	0.048	0.028	0.706†	1			
Pyridinoline (ng/mL)	0.051	0.063	0.004	-0.013	1		
Deoxypyridinoline (nmol/mL)	0.080	0.126*	0.048	-0.004	0.269†	1	
TRACP-5b (U/L)	0.176†	-0.081	0.120	0.110	-0.017	-0.064	1
UHYP (µg/g creatinine)	0.258†	-0.091	0.068	0.101	0.003	0.028	0.265†

*p<0.05; †p<0.01
BLL: Blood lead level; OC: Osteocalcin; ALP: Alkaline phosphatase; BALP: Bone-specific alkaline phosphatase; PYD: Pyridinoline;
DPYD: Deoxypyridinoline; TRACP-5b: Tartarate-resistant acid phosphatase-5b; UHYP: Urinary hydroxyproline

Table 4: Bone turnover markers levels in different groups of workers stratified by blood lead level and work experience (level of exposure)

Independent variable	n	Bone formation biomarkers			Bone resorption biomarkers			
		OC (ng/mL)	ALP (U/L)	BALP (U/L)	PYD (ng/mL)	DPYD (nmol/mL)	TRACP-5b (U/L)	UHYP (µg/g Cr)
Blood lead level (µg/dL)								
≤15	51	16.6 (8.9)	90 (26)	37 (20)	33 (15)	0.237 (0.700)	3.0 (2.0)	5.0 (3.9)
16–25	76	16.6 (9.8)	92 (23)	40 (22)	33 (17)	0.253 (0.900)	3.4 (2.3)	5.8 (4.7)
26–35	58	14.2 (7.1)	90 (26)	41 (25)	34 (14)	0.239 (0.700)	3.0 (2.0)	5.3 (2.5)
36–45	40	12.9 (4.4)	90 (28)	41 (22)	32 (10)	0.234 (0.800)	3.8 (2.5)	7.8 (5.4)
>45	31	18.5 (12.0)	92 (34)	41 (26)	36 (17)	0.239 (0.700)	4.1 (2.2)	5.4 (3.2)
p value (ANOVA)		0.040	0.982	0.897	0.841	0.446	0.052	0.015
Exposure (yrs)								
≤5	85	15.7 (4.7)	88 (23)	35 (14)	31 (9)	0.237 (0.400)	2.5 (2.3)	3.5 (2.4)
6–10	30	17.9 (11.0)	99 (32)	48 (30)	36 (23)	0.232 (0.700)	4.1 (2.6)	7.3 (7.0)
11–15	100	15.6 (11.0)	92 (27)	41 (26)	35 (18)	0.255 (0.900)	3.8 (2.3)	5.3 (5.0)
>15	41	14.5 (9.4)	89 (27)	41 (23)	34 (10)	0.212 (0.700)	3.4 (2.0)	4.8 (4.0)
p value (ANOVA)		0.458	0.275	0.037	0.363	0.016	<0.001	0.002

OC: Osteocalcin; ALP: Alkaline phosphatase; BALP: Bone-specific alkaline phosphatase; PYD: Pyridinoline; DPYD: Deoxypyridinoline; TRACP-5b: Tartarate-resistant acid phosphatase-5b; UHYP: Urinary hydroxyproline

group.[37] BTM has been used for the assessment of early stage osteoporosis.[38] Animal studies of lead intoxication have reported higher lead accumulation in osteoblast cultures,[39] decreased bone density,[40] delayed fracture healing,[21] and increased BTMs[41]. The changes of bone formation and bone resorption markers lead to osteocalcin-dependent osteopenia.[42]

Workers with occupational exposure to lead have reported deteriorated bone mineral density and osteoporosis.[26,27] General population with higher urinary lead levels has increased odds of osteopenia and osteoporosis.[43] Potula, *et al,*[24] reported significantly higher levels of bone resorption markers (UPYD and UDPYD) in women engaging in lead-smelting process. Sun, *et al,* also reported significant higher levels of BALP, OC and UHYP in lead-battery workers.[29] In the current study, we noted a significantly higher bone formation and bone resorption markers in male workers with chronic exposed to lead in a lead-battery manufacturing plant.

An animal study indicated that long-term lead-exposure causes deterioration of bone microstructures.[44] We found that the bone resorption biomarkers were significantly affected. Sun, *et al*, also reported a positive and significant association between BLL and bone biomarkers (OC, BALP, and UHYP) in lead-battery workers.[29] We found that BLL had a significant negative correlation with osteocalcin and a significant positive correlation with TRACP-5b and UHYP. The bone formation biomarkers (*eg*, BALP) and bone resorp-

tion biomarkers (*eg*, DPYD, TRACP-5b, and UHYP) were significantly associated with the duration of exposure. In conclusion, long-term exposure to lead results in high BLLs and altered BTM.

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