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Original Research

Assessment of the relationship between blood lead levels and hematological parameters among lead acid –storage battery plant workers

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Abstract
The present study investigated the relationship between blood -lead levels and hematological parameters and urinary- δ -ALA levels in 391 male lead acid –storage battery plant workers. Blood lead levels (BLL) done by using an atomic absorption spectrophotometer. Hematological parameters were done by using the ABX Micros ES -60 hematology analyzer. The reticulocyte counts in subjects were done by using the supra-vital staining method. Urinary- δ -ALA levels were determined by using spectrophotometric method. The levels of BLL ($p<0.0001$) and urine – δ-ALA ($p<0.0001$) were considerably increased in the high lead exposed workers as compared to low lead exposed workers. The reticulocyte counts and urinary- δ -ALA were positively and significantly associated with lead in blood of subjects with > 30µg/dL. The present study suggests that levels of reticulocyte count and urinary- δ -ALA could be used as bio-markers to control the occupational exposure of lead.

INTRODUCTION

The manufacturing process of lead –acid battery industries involve: lead oxide manufacture, grid casting, pasting, plate cutting, formation, charging and assembly. The hazardous chemicals used during this process lead oxide (PbO_2), spongy lead (Pb) and sulphuric acid (H_2SO_4). The workers engaged in those processes are exposed to lead through inhalation, ingestion and dermal contact. The lead accumulation occurs in red blood cells, soft tissue (brain, kidney & bone marrow) and mineralized tissue (bone & teeth). Air lead levels in lead acid- battery manufacturing areas are associated with increased BLL [1]. The chronic exposure to lead from lead acid–battery manufacturing process has reported a variety of health effects such as dental health [2-3], hematological [4-5], Matrix –gamma-carboxy glutamic acid protein (MGP) polymorphism [6], risk of cardiovascular diseases [7], kidney and liver [8-10], oxidative stress [11-12], pteridine metabolism with neurotransmitters synthesis [13], immunological [14], genotoxicity [15], reproductive [16], neuropsychological [17], monoamine metabolites [18], Delta-aminolevulinic acid dehydratase gene polymorphism with neurobehavioral function [19] and bone mineral density [20].

The study included the biological monitoring in workers exposed to Pb from the lead-battery manufacturing process. It included processes, estimation of blood - lead levels, urine- δ-aminolevulinic acid and hematological parameters. In addition, serum creatinine and blood urea nitrogen were estimated and to explore the association between bloods lead levels and urine-δ-ALA as surrogate biomarker for lead exposure.

MATERIAL AND METHODS

The present study was carried out at lead battery plant located at Tamilnadu, India. A total of 391 male workers from 8 different sections participated in the study. The study subjects were categorized into two groups according to Biological Exposure Indices (BEI) of American Conference of Governmental Industrial Hygienist (ACGIH-2011) [21]. The first group consisted of 247 (63%) workers with their BLL were ≤ 30 µg/dL and considered them as low lead exposure and the second group comprise of 144 (37%) workers with their BLL were > 30 µg/dL and consider as high lead exposure. Informed consent was taken from each subject for participation in the study. Ethical committee at the centre permitted to conduct the experiment on these subjects.

***Medical and demographic information*:** The socio-demographic characteristics, personal habits like alcohol consumption, smoking, dietary pattern, place of residence and occupational history related to lead exposure were recorded in the pre-designed questionnaire. Body mass index (BMI) was calculated and expressed as Kg/m^2.

Blood lead level: Three ml of venous whole blood was collected in heparinized vacuette from the study subjects and were stored at -20º C until the analysis. Two ml of whole blood was digested by using ETHOS -D, Milestone Microwave laboratory systems (Italy) with 2 ml of Nitric Acid (HNO_3) and 0.2 ml of hydrogen peroxide (H_2O_2) with maintaining of power, temperature and duration of time. The digested samples were made up to 5 ml using triple distilled water and centrifuged. The concentration of lead was measured by using Atomic Absorption Spectrophotometer GBC-Avanta, Australia. A known concentration of lead standard solution was digested and analyzed for internal quality control. The recovery rate of 200 µg/L of lead solution was found in 101% with RSD of less than 0.5%. The blood -lead level was expressed as µg/dL.

***Urinary-δ-Aminolevulinic acid*:** Urine samples were collected from study subjects using polypropylene containers, which contained 2 grams of barbituric acid as preservative. The urine samples were refrigerated at 4^oC until the analysis. The urinary-δ -ALA levels were determined by the method of Katsumaro and Masana (1972) [22]. Urine samples were heated with buffered ethyl acetoacetate to produce pyrrole derivative. This derivative was extracted into ethyl-acetate and added modified Ehrlich's reagent to produce a reddish color. After 10 minutes, the absorbance of the color solution was recorded at 550nm by using the spectrophotometer. The urinary-δ -ALA was measured from a standard curve prepared by using an aqueous standard solution of 0-30 mg/L ALA. The levels of urinary-δ -ALA was expressed as mg/L.

Hematological examination: 2 ml of venous whole blood was collected in lavender topped vacutainer tube, which containing tri-potassium–EDTA. The levels of RBC count, hemoglobin (Hb) and hematocrit (Hct) were done by using an ABX Micros ES -60 hematology analyzer. The instrument was calibrated by using Bio-Rad QC (Lot No.76752) before analysis for the samples. The values of red blood indices such as MCV, MCH and MCHC were calculated by using RBC count, hemoglobin and heamtocrit levels within the sample.

Reticulocyte count: Non-nucleated immature red cells contain nuclear remnants of RNA known as a reticulocyte. The reticulocyte counts in peripheral blood samples were done using the supra-vital staining method. The reticulocyte counts in study subjects were expressed as a percentage.

Renal function tests: Serum creatinine and blood urea nitrogen in study subjects were estimated by using Bayer diagnostic kits and Random Access analyzer, and expressed as mg/dL.

***Statistical analysis*:** Software package SPSS, version 17.0 for windows were used in analysis of the data. The student t-test was used to compare different parameters of lead -exposed workers. Pearson's correlation test was used to find out the association between blood -lead levels and hematological parameters and urinary-δ-ALA in lead exposed workers.

RESULTS

The demographic details of age, experience, BMI and frequency distribution of lifestyle confounding factors of low and high lead exposed workers were reported in Table 1.

Table 1. Demographic detail of workers exposed to lead

Parameters	Low exposure (n=247)	High exposure (n=144)
Age(years)	35.1 ± 4.1[a]	35.1 ± 4.1
Experience(years)	11.8 ± 2.7	10.4 ± 3.4
BMI(Kg/m^2)	24.9 ± 2.6	24.8 ± 2.8
Smoking		
Yes	44(18)[b]	32(22)
No	203(82)	112(78)
Alcohol consumption		
Yes	79(32)	66(46)
No	168(68)	78(54)

a= mean ± standard deviation

b= Number & parenthesis indicates %

Table 2. Blood lead levels in workers according to sections in lead acid storage manufacturing plant

Sections	(n=391)	Mean ± SD	>30 (µg/dL)
Pasting	42	35.9 ± 10.4	31(74)[a]
Casting	46	26.0 ± 9.6	10(22)
Oxide Mill	09	30.1 ± 8.5	04(44)
Assembly	215	29.0 ± 11.7	87(40)
Plate cutting	04	30.0 ± 4.2	01(25)
Charging	25	19.7 ± 9.3	02(08)
Formation	08	16.4 ± 4.5	00(0)
Maintaince	42	22.0 ± 8.3	09(21)

a=Number and parenthesis indicates %

Blood lead levels in workers according to different sections of lead battery plant were reported in Table-2. The highest BLL was noticed in pasting section area workers with 74% had > 30 µg/dL. The oxide mill and assembly section workers showed the similar percentage as the increase.

The levels of BLL, urinary- δ-ALA, hematological parameters in lead exposed workers presented in Table-3. The mean levels of BLL (P<0.0001) and urinary- δ -ALA (P<0.0001) were significantly higher in the high lead exposed workers as compared to low lead exposed workers. The hematological parameters did not show any consequential differences between two groups.

The levels of serum creatinine and blood urea nitrogen in lead exposed workers were presented in Table-4. The renal function tests showed no significant increase in the high lead exposed workers as compared to low lead exposed workers.

Correlation between BLL and hematological examination in the high lead exposed workers were presented in Table-5. The BLL showed positive and significant (p<0.01) correlation with reticulocyte counts in workers with their BLL were > 30 µg/dL.

The relationship between BLL and urinary- δ -ALA levels in the low and high lead exposed workers presented in Figure-1, showed positive correlation and significant (R2= 0.135).

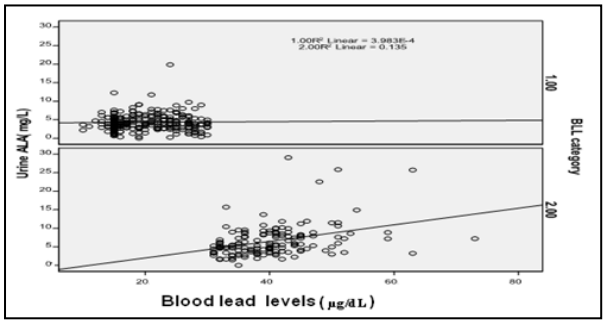

Figure 1. Relationship between BLL and urine -δ- ALA levels in lead exposed workers.

Table 3. Blood lead, hematological & urine δ-ALA levels in workers exposed to lead

Parameters	Low exposure (n=247)	High exposure (n=144)	p (value)
Blood lead levels(µg/dL)	20.1 ±5.1	40.37 ±7.2*	0.0001
Urine □-ALA(mg/L)	4.3 ± 2.2	6.5 ± 4.4*	0.0001
Red blood cells (Cell/cumm)	5.3 ± 0.4	5.3 ± 0.4	0.622
Hemoglobin (g/dL)	14.8 ± 1.1	14.8 ± 1.0	0.916
Hematocrit (%)	44.8 ± 3.0	44.9 ± 3.1	0.591
MCV	84.7 ± 9.3	84.2 ± 5.5	0.509
MCH	28.0 ± 3.5	27.7 ± 2.2	0.327
MCHC	33.0 ± 1.2	33.0 ± 0.8	0.345
Reticulocyte count (%)	1.0 ± 0.3	1.0 ± 0.3	0.757

*P<0.0001

Table 4. Renal function tests in lead exposed workers

Parameters	Low exposure (n=247)	High exposure (n=144)	p (value)
Serum creatinine (mg/dL)	1.0 ± 0.3	1.0 ± 0.2	0.590
Blood urea nitrogen (mg/dL)	11.6 ± 3.3	11.6 ± 3.2	0.980

DISCUSSION

The study examined the effect of occupational lead exposure on hematological parameters and urinary- δ -ALA changes among 391 male workers from 8 different sections of a lead-acid storage manufacturing process. In the present study, we observed that the 63% of workers had BLL ≤30 µg/dL and 37% worker had > 30 µg/dL. It also noticed that BLL was decline in the following order: pasting, oxide mill, assembly, plate cutting, casting, maintaince, charging and formation. US EPA [23] has reported that highest emission of lead was noticed in pasting section area followed by grid casting, oxide mill and three process operations such as plate stacking, plate burning & assembly. During the study, we noticed a highest BLL in pasting section workers followed by oxide mill, plate cutting and assembly section workers from the plant. Average worker BLL was reported in developing countries was 47 µg/dl [24]. In the present study, we reported average BLL in pasting area workers was 36 µg/dl and lowest average BLL was reported in formation area workers with 16 µg/dl. The average BLL reported in this study were similar to the workers involved in Taiwan battery manufacturing plant [25].

Table 5. Correlation coefficients (r) between blood lead levels and hematological parameters in high exposure group

Variable	BLL (µg/dL)	Hb %	RBC (cell/cu mm)	Hematocrit (%)	Reticulocyte (%)
B-Pb (µg/dL)	1.000	-	-	-	-
Hb%	0.103	1.000	-	-	-
RBC (cell/cu mm)	0.100	0.454**	1.000	-	-
Hematocrit (%)	0.092	0.934**	0.619	1.000	-
Reticulocyte (%)	0.215**	0.136	0.017	0.118	1.000

**Correlation is significant at the 0.01 level

Patil et al. [11] and Fonte et al [5] have reported significantly decreased hematological parameters in lead exposed workers with average BLL 54 and 148 µg/dl respectively. In the present study, no significant decrease was noticed in the high lead exposed workers as compared to low lead exposed workers. The average BLL observed in this study was 47 µg /dL.

Tian et al [26] reported a dose- response relationship between BLL and urinary albumin and N-acetyl-D-glucosamindase in lead battery workers. Santos et al [27], Omae et al [28] and Wang et al [9] observed that the BLL higher than 60 µg /dl had no chance of renal dysfunction. In the present study, we reported no abnormal renal function in high lead exposed workers, because the average BLL in the high lead exposed workers was 47 µg /dL.

Most of the studies reported a positive & significant association between BLL and urinary- δ -ALA in lead battery factory workers [29-32]. In the present study, we noticed a positive & significant association between BLL and urinary- δ -ALA in workers BLL was > 30 µg /dL.

Conclusion: Blood lead levels were significantly increased in the high lead exposed workers as compared to low lead exposed workers. The reticulocyte counts and urinary- δ -ALA levels were positively and significantly associated with BLL of workers greater than 30µg/dL.

REFERENCES

1. Ibiebele DD. Air and blood lead levels in a battery factory. Sci Total Environ. 1994, 152(3):269-73.
2. Suyama Y, Takaku S, Okawa Y, Matsukubo T.Dental erosion in workers exposed to sulfuric acid in lead storage battery manufacturing facility. Bull Tokyo Dent Coll. 2010; 51(2):77-83.
3. Bamise CT, Esan TA, Ajayi JO, Olagundoye O, Oziegbe EO. Dental erosion in a road-side battery technician: case report and a review of the literature. Oral Health Prev Dent. 2008; 6(3):249-53.
4. Shaik AP, Jamil K.A study on the ALAD gene polymorphisms associated with lead exposure. Toxicol Ind Health. 2008 24(7):501-6.
5. Fonte R, Agosti A, Scafa F, Candura SM. Anemia and abdominal pain due to occupational lead poisoning. Haematologica. 2007; 92(2):e13-4.
6. Shaik AP, Jamil K.Polymorphisms in MGP gene and their association with lead toxicity. Toxicol Mech Methods. 2009; 19(3):209-13.
7. Kim KR, Lee SW, Paik NW, Choi K.Low-level lead exposure among South Korean lead workers, and estimates of associated risk of cardiovascular diseases. J Occup Environ Hyg. 2008 Jun;5(6):399-416.
8. Patil AJ, Bhagwat VR, Patil JA, Dongre NN, Ambekar JG, Das KK.Occupational lead exposure in battery manufacturing workers, silver jewelry workers, and spray painters in western Maharashtra (India): effect on liver and kidney function. J Basic Clin Physiol Pharmacol. 2007; 18(2):87-100.
9. Wang VS, Lee MT, Chiou JY, Guu CF, Wu CC, Wu TN, Lai JS.Relationship between blood lead levels and renal function in lead battery workers. Int Arch Occup Environ Health. 2002 Oct;75(8):569-75.
10. Ehrlich R, Robins T, Jordaan E, Miller S, Mbuli S, Selby P, Wynchank S, Cantrell A, De Broe M, D'Haese P, Todd A, Landrigan P.Lead absorption and renal dysfunction in a South African battery factory. Occup Environ Med. 1998;55(7):453-60.
11. Patil AJ, Bhagwat VR, Patil JA, Dongre NN, Ambekar JG, Jailkhani R, Das KK.Effect of lead (Pb) exposure on the activity of superoxide dismutase and catalase in battery manufacturing workers (BMW) of Western Maharashtra (India) with reference to heme biosynthesis. Int J Environ Res Public Health. 2006;3(4):329-37.
12. Gurer-Orhan H, Sabir HU, Ozgüneş H.Correlation between clinical indicators of lead poisoning and oxidative stress parameters in controls and lead-exposed workers. Toxicology. 2004 Feb 15; 195(2-3):147-54.
13. Engin AB, Tuzun D, Sahin G.Evaluation of pteridine metabolism in battery workers chronically exposed to lead. Hum Exp Toxicol. 2006; 25(7):353-9.
14. Queiroz ML, Perlingeiro RC, Bincoletto C, Almeida M, Cardoso MP, Dantas DC.Immunoglobulin levels and cellular immune function in lead exposed workers.

Immunopharmacol Immunotoxicol. 1994; 16(1):115-28.

15. Duydu Y, Süzen HS, Aydin A, Cander O, Uysal H, Işimer A, Vural N.Correlation between lead exposure indicators and sister chromatid exchange (SCE) frequencies in lymphocytes from inorganic lead exposed workers. Arch Environ Contam Toxicol. 2001; 41(2):241-6.
16. Robins TG, Bornman MS, Ehrlich RI, Cantrell AC, Pienaar E, Vallabh J, Miller S.Semen quality and fertility of men employed in a South African lead acid battery plant. Am J Ind Med. 1997; 32(4):369-76.
17. Kumar P, Husain SG, Murthy RC, Srivastava SP, Anand M, Ali MM, Seth PK.Neuropsychological studies on lead battery workers. Vet Hum Toxicol. 2002; 44(2):76-8.
18. Tang HW, Liang YX, Hu XH, Yang HG. Alterations of monoamine metabolites and neurobehavioral function in lead-exposed workers. Biomed Environ Sci. 1995; 8(1):23-9.
19. Gao A, Lu XT, Li QY, Tian L.Effect of the delta-aminolevulinic acid dehydratase gene polymorphism on renal and neurobehavioral function in workers exposed to lead in China. Sci Total Environ. 2010; 408(19):4052-5.
20. Raafat BM, Hassan NS, Aziz SW. Bone mineral density (BMD) and osteoporosis risk factor in Egyptian male and female battery manufacturing workers. Toxicol Ind Health. 2012;28(3):245-52.
21. American Conference of Governmental Industrial hygienist (2011): The documentation of threshold limit values and Biological exposure indices of chemical and physical agents. Cincinnati, USA.
22. Katsumaro Tomokuni and Masana Ogata. Simple Method for Determination of Urinary –Aminolevulinic acid as an Index of Lead Exposure. Clin Chem, 1972; 18(12): 1534-1536.
23. Lead acid battery manufacture-Background information for proposed standards, EPA 450/33-79-028a, US EPA, Research triangle park, NC, November 1979.
24. Gottesfeld P and Pokhrel AK. Review: Lead exposure in battery manufacturing and recycling in developing countries and among children in nearby communities. J Occup Environ Hyg. 2011;8(9):520-32.
25. Chuang HY, Cheng WC, Chen CY, Yang YH, Sung FC, Yang CY, Wu TN.A follow-up comparison of blood lead levels between foreign and native workers of battery manufacturing in Taiwan. Sci Total Environ. 2008; 394(1):52-6.
26. Tian LT, Lei LJ, Chang XL, Jin TY, Zheng G, Guo WJ, Li HQ, Pan XH. Risk assessment of renal dysfunction caused by occupational lead exposure. Zhonghua Lao Dong Wei Sheng Zhi Ye Bing Za Zhi. 2010 ;28(3):170-4
27. Dos Santos AC, Colacciopo S, Dal Bó CM, dos Santos NA. Occupational exposure to lead, kidney function tests, and blood pressure. Am J Ind Med. 1994; 26(5):635–643.
28. Omae K, Sakurai H, Higashi T, Muto T, Ichikawa M, Sasaki N. No adverse effects of lead on renal function in lead-exposed workers. Ind Health. 1990; 28(2):77–83.
29. Vinod.R.Bhagwat, Arun J.Patil, Jyotsna A. Patil and Ajit V. Sontakke. Occupational lead exposure and liver functions in battery manufacture workers around Kolhapur (Maharashtra). Al Ameen J Med Sci. 2008; 1 (1):2 -9.
30. Kae Higashikawa, Katsuya Furuki, Shiro Takada, Satoru Okamoto, Hiroshi Ukai, Takashi Yuasa And Masayuki Ikeda. Blood lead level to induce significant increase in urinary δ-aminolevulinic acid level among lead-exposed workers: a statistical approach Industrial health 2000, 38, 181–188
31. Makino S, Hiroshi Tsuruta and Tsutomu Takata. Relationship between blood lead level and urinary ALA level in workers exposed to very low levels of lead. Industrial Health 2000, 38, 95–98
32. Fukui Y, Miki M, Ukai H, Okamoto S, Takada S, Ikeda M. Comparison of colorimetric and HPLC methods for determination of delta-aminolevulinic acid in urine with reference to dose-response relationship in occupational exposure to lead. Ind Health. 2005; 43(4):691-8.