Original Article

LEAD AND ESSENTIAL TRACE ELEMENT LEVELS IN ACID BATTERY MANUFACTURING AND AGRICULTURE WORKERS:A CROSS SECTIONAL STUDY

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ABSTRACT

Lead (Pb) has plethora of industrial applications, and has known to for its deleterious effects on various organs. In the current study, we aimed to assess the occupational Pb exposure and relationship with serum creatinine, cholesterol & pre-existing nutritional status, among 'acid-battery industry' (Group-A, n=110), and 'agriculture' (Group-B, n=50) workers. Blood Lead Levels (BLL), trace elements (Fe, Zn, Cu, Mg & Ca), serum creatinine and cholesterol levels were assessed. The BLL in group-A were significantly ($p<0.01$) higher than group-B participants with mean BLL being 94.0±40.47 and 11.2±7.85 µg/dL respectively. Serum creatinine and cholesterol levels were significantly higher in group-A subjects than group-B. Further, elevated BLL of group-A have correlated positively ($p<0.01$) with serum creatinine ($r = 0.333$) and inversely with serum albumin ($r = -0.240$), Fe ($r = -0.453$), Cu ($r = -0.303$) & Ca ($r = -0.193$; $p<0.05$). The participants from group-A were referred to 'Chelation therapy (BLL > 80µg/dL)' and temporarily shifted to 'no-Pb exposure' work. The results suggest that group-A had elevated BLL due to persistence exposure to Pb at work place. Further, nutritional and trace element status might have limited effect on elevated BLL in 'chronic high level exposure'. Regular BLL screening and preventive measures are mandatory to reduce the risks associated with work place Pb exposure.

Keywords: blood lead levels; occupational exposure; serum trace elements; serum cholesterol; creatinine levels.

INTRODUCTION

Lead (Pb) is a soft metal, which has many known applications over the decades. The unique properties of Pb such as low melting point, ability to form carbon metal compound, holds pigment well, recyclability and corrosion resistance made it useful in industrial applications (ATSDR 2005; Dongre et al., 2010). The tetra-ethyl Pb was used in gasoline as anti-knocking agent, but recently is being phased out with the unleaded-gasoline in several countries (Kovarik 2005). Another important and unavoidable application of Pb is in "acid battery industry", where majority of the Pb was recycled manually. According to 'United Nations Environment Programme-2008', 78% of worldwide production of Pb was consumed in 'Pb acid batteries'. The prevalence of work place exposure to Pb is higher in acid battery industry employees specially in developing nations like India, where poor hygiene and inappropriate safety measures are being practiced (Bhagwat et al., 2008). The toxic effects of Pb on haemopoietic, renal and central nervous systems have been reported over the years (ATSDR 2005; UNEP 2008; Granick et al 1972). In addition, it has been well documented that inhibition of δ-Aminolevulinic acid dehydratase activity, increased N-acetyl-D-glucosaminidase activity and cognitive function evaluation by learning and memory tests were considered as the biomarkers for Pb toxicity on haemopoietic, renal and central nervous system, respectively (ATSDR 2005; UNEP 2008; Chia et al 1994; Flora et al., 2006). These surrogate markers are sensitive and reliable for 'early detection of sub-clinical Pb toxicity especially in growing children', but dose-response relation between Pb and these markers is limited in chronic high level exposure such as occupationally exposed adults. Further, the toxic effects of Pb on other organ systems viz kidney and liver are sparsely available with reference to occupationally exposed groups. The recent reports suggest that liver and kidney functions get altered in the Pb exposed individuals (Dongre et al., 2010, Bhagwat et al., 2008). Autopsy studies of Pb exposed humans indicated that majority

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(33%) of Pb was found in liver followed by kidney cortex and medulla (Madipalli 2007).

Accumulating evidences suggest that nutritional status interact with toxicants and thus modulate the susceptibility to toxicity. The essential trace elements such as iron (Fe), zinc (Zn), copper (Cu), magnesium (Mg), calcium (Ca) etc. play an important role to combat the Pb toxicity. Studies in rat models demonstrated that trace elements such as Ca, Fe, Zn modulate susceptibility to Pb toxicity (Ettinger et al., 2007; Jamieson et al., 2006). Further, human studies demonstrate association of Fe, Mg status with blood Pb levels and toxicity (Anetor et al., 2007, Jain et al., 2005, Muwakkit et al., 2008). It is thought that pre-existing serum trace element levels may influence the absorption, distribution and toxic effects of Pb. Further, our previous observation in school children residing at urban industrial areas and buffaloes from sub-urban areas have shown a significant inverse relationship between serum Fe and blood Pb levels of as low as 20 µg/dL (Reddy et al., 2011; Shailaja et al., 2014). It has been well reported that industrialization, urbanization and anthropogenic activities led to elevated 'blood lead levels' (BLL >20 µg/dL) in population residing in industrial, urban and semi-urban areas than remote rural areas (ATSDR 2005; Dongre et al., 2010; Reddy et al., 2011).

With reference to the above observations the present study was carried out with an aim "to determine Pb and essential trace element levels" in acid battery industry employees from Hyderabad and agriculture workers from remote rural areas of Andhra Pradesh, India. In addition, the relationship of BLL with serum creatinine, cholesterol and nutritional status were also assessed.

MATERIAL AND METHODS

Study Design and Location

A cross-sectional study was carried out among persons involved in Pb acid battery manufacturing industry (Group-A) and agriculture workers (Group-B) from Andhra Pradesh, India. The battery industry was located in sub-urban region of Hyderabad city, Andhra Pradesh, India. The enrollment of 'administrative staff' as 'control subjects' was ruled out, keeping in view of our previous observation of higher BLL (>20 µg/dL) in school children residing at urban industrial area. Further as low as 20 µg/dL BLL have shown inverse relationship with serum Fe levels. So, the controls were the agriculture workers, who were residing in remote rural area situated far away from urbanized and industrialized society.

Selection of Subjects

The sample size was calculated considering the variation (5.25 µg/dL) in the blood Pb levels of previous study conducted in occupational group (George Foundation 1999), with a Confidence Interval of 95% and 0.8 precision, a sample size of 110.30 'Pb acid battery industry employees' was arrived and rounded off to 110. A total of 110 male subjects were enrolled out of the 216 employees, who were actively engaged in Pb smelting and packing process, from the same acid battery industry, to minimize the variation of exposure to Pb. The study subjects were apparently healthy, non-smoking and non-alcoholic (at least since 12 months) individuals, aged between 25 – 45 years with a minimum work experience of 4 – 6 years. Similarly, age-matched male agriculture workers (n=50) from remote rural area were enrolled in the study as the control subjects.

The individuals with infections or any other illness were excluded from the study, by the physician at the time of sample collection. The information on work load such as work burden, working hours per day, week and month were collected. The anthropometric parameters such as height and weight were recorded. Systolic and diastolic blood pressures were recorded using mercury sphygmomanometer. The study and procedures were approved by Institutional Review Board (Institutional Ethics Committee for Human Studies) of National Institute of Nutrition (ICMR), Hyderabad, India. As per the recommendations of IRB a written consent was obtained from all the participants.

Sample Collection and Laboratory investigations

About 5.0 mL of blood was collected from all the participants by veni-puncture in two vacutainer tubes for whole blood and serum. Serum albumin, total protein, creatinine and cholesterol levels were determined by ACETM clinical autoanlyzer (Model: Ace Alera). Blood Pb and serum trace element levels were estimated in "microwave digested samples" as explained by Borowski & Schmaling (Borowski & Schmaling, 1999). Briefly, 1.0 mL of blood and 0.5 mL of serum were digested with 2.0 mL of ultrapure nitric acid (Merck, Mumbai, India) in a closed microwave (Marsxpress, CEM Corp., Matthews, USA) digestion system. Blood Pb level was measured on

Graphite Furnace Atomic Absorption Spectrophotometer (GF-AAS, Thermoelectron) with a detection limit of 0.321 µg/L. Two levels of 'certified reference blood Pb standards' obtained from ESA Inc., USA (Level 1 : 7.1±3.0 µg/dL, Lot No. 082306 and Level 2 : 27.2±4.0 µg/dL, Lot No. 022406) were analyzed simultaneously 'to ensure quality of estimates' and noted 96-98% of recovery. Serum Fe, Zn, Cu, Mg and Ca levels were measured using flame-AAS (VARIAN 220, Pala Alto, CA, USA). The prevalence of micronutrient deficiencies was computed considering the cut-off values of serum Fe (<70 µg/dL), Zn (<70 µg/dL), Cu (<80 µg/dL), Mg (<1.8 mg/dL), Ca (<8.0 mg/dL) as described previously (Tietz, 1986).

Statistical Analysis

The BLL data was log transformed to avoid false positive results, as there was a wide variation in BLL. Mean and standard deviation (SD) were calculated for all the variables. The student t-test was applied to assess the statistical difference in mean levels of various parameters between two groups. Relationships were identified by means of Pearson's bivariate moment correlation coefficient. The results were considered significant at $p < 0.05$. The data were analyzed using SPSS 15.0 Window's version.

RESULTS

The mean age (Group-A 32.1±8.51 years and Group-B 34.8±6.25 years), height, weight and BMI of the participants from both the groups were comparable. The systolic and diastolic blood pressures of group-A participants were 125.4±12.68 and 86.2±6.54 mm of Hg; similarly the group-B participants had 128.3±18.84 and 80.4±9.16 mm of Hg as systolic and diastolic blood pressures. The diastolic blood pressures of group-A were significantly ($p<0.05$) higher as compared to group-B, whereas the systolic pressures were comparable between the groups. The group-A employees have 8.0 h work shift per day, six days a week, whereas group-B individuals have a history of 6 – 7 h work period per day, six to seven days per week during the monsoon and agriculture farming periods.

Biochemical Parameters

The serum albumin, total protein, creatinine and cholesterol levels of all the participants were given in Table – 1. Serum albumin and total protein levels were significantly ($p<0.05$) low in group-B as compared to group-A. Serum creatinine levels, an indicator of kidney function, were significantly ($p<0.05$) elevated in group-A individuals as compared with group-B. Similarly, serum cholesterol levels were also elevated in group-A than group-B participants (Table 1).

Table 1: Clinical chemistry profile of participants.

S. No.	Parameter	Group – A (n=110)	Group – B (n=50)
1	Serum Albumin (g/dL)	4.0±0.77	2.9 ±0.83*
2	Total Protein (g/dL)	7.8±0.93	6.4±2.02 *
3	Serum Creatinine (mg/dL)	1.23±077	1.07±0.673*
4	Cholesterol (mg/dL)	155.9±42.65	104.2±48.33*

Values represent mean±SD of variables.

* Indicates significantly different at $p<0.05$.

Blood Lead Levels BLL and Serum Trace Element Levels

A wide variation in Blood lead levels (BLL) was noted among the workers of acid battery industry. Blood lead levels ranged from 18.3 µg/dL to 209 µg/dL and from 2.25 µg/dL to 20.5 µg/dL in group-A and group-B subjects respectively. The BLL in group-A was significantly ($p<0.01$) higher than in group-B participants with mean BLL being 94.0±40.47 and 11.2±7.85 µg/dL, respectively (Table 2). Mean serum trace element levels of group-A and group-B subjects were given in Table 2. The serum Fe levels ranged from 65.0 – 288.3 and 64.2 – 198.0 µg/dL in group-A and group-B participants respectively. Mean serum iron levels were significantly ($p < 0.05$) low in group-B (113.0 ± 30.51 µg/dL) than group-A (181.8 ±57.72 µg/dL). The serum Zn levels in group-A was ranged from 65.3 – 178.5 µg/dL and in group-B was 66.4 – 130.2 µg/dL. Mean serum zinc levels were also significantly ($p<0.05$) lower in group-B (66.4±15.10 µg/dL) than group-A (112.5±20.21 µg/dL) individuals. Serum copper levels ranged from 68.6 to 148.3 and 46.7 to 180.0 µg/dL in group-A and group-B respectively. The serum magnesium levels in group-A and group-B were ranged from 1.2 to 3.8 and 1.6 to 2.8 mg/dL, respectively.

Serum calcium levels were ranged from 4.7 to 11.9 and 4.2 to 12.3 mg/dL in group-A and group-B, respectively. Therewas no significant difference between mean serum Cu, Mg and Ca levels among the two groups.

Table 2: Blood Lead and Serum Trace Element Levels

S. No.	Parameter	Group – A (n=110)	Group – B (n=50)
1	Blood Lead Level (µg/dL)	94.0±40.47	11.2±7.85**
2	Fe (µg/dL)	181.8±57.72	113.0±30.51*
3	Zn (µg/dL)	112.5±20.21	66.4±15.10 *
4	Cu (µg/dL)	98.9±1.86	108.0±18.31
5	Mg (mg/dL)	2.03±0.208	2.50±0.670
4	Ca (mg/dL)	9.0±1.45	8.5±2.66

Values represent mean±SD of variables.

* Indicates significantly different at p <0.05.

** Indicates significantly different at p <0.01.

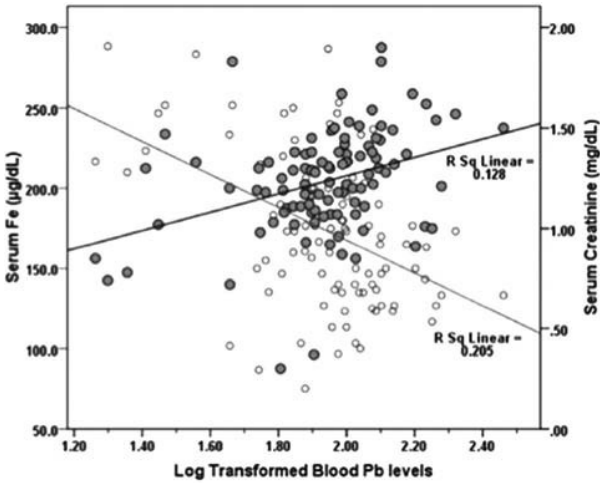
Prevalence of Pb Toxicity and deficiency of trace element levels among participants

The participants of group-A were categorized based on blood lead level cut-offs specified by various organizations (Table 3). Only three percent of the participants from group-A had the BLL <25 µg/dL, cut-off specified by 'Adult Blood Lead Epidemiology and Surveillance' (ABLES) program managed by CDC's National Institute for Occupational Safety and Health (NIOSH) (CDC-MMWR, 2014); whereas 6% of the participants had the BLL <30 µg/dL, the limits specified for Biological Exposure Indices (BEI) by American Conference of Governmental Industrial Hygienists (ACGIH, 2012). Ninety four percent of group-A subjects had BLL above 40.0 µg/dL, the safe cut-off limit set by CDC, Atlanta; OSHA and WHO for the work place exposure (CDC-MMWR, 2014). It was alarming to note that 33% of group-A participants had mean BLL above 100 µg/dL (136.2±38.22 µg/dL) in (Table 3). Whereas, the group-B subject's mean blood lead levels (11.2±7.85 µg/dL) were within the specified cut-off limits. Ten percent of group-A and 35% of group-B individuals had serum Fe deficiency. Similarly, serum Zn was deficient in 12% of group-A and 28.9% of the group-B individuals. The serum copper deficiency was observed in 13.0% of the group-A and 26.32% of group-B individuals. Similarly, serum Ca was deficient in 18% and 22.0% of group-A and group-B subjects respectively. Serum Mg deficient in 3.7 % of group-A and 11.2% of group-B participants.

Correlation of Blood Lead Levels with Clinical parameters and Trace Elements

Elevated blood Pb levels of group-A participants had shown significant ($p<0.01$) positive correlation with serum creatinine ($r = 0.333$) (Fig. 1) and an inverse correlation with serum albumin levels ($r = -0.240$).. However, no such correlation was observed between elevated BLL and serum total protein, cholesterol levels among group-A individuals. Further, the elevated BLL in group-A have shown significant ($p < 0.01$) inverse correlation with serum Fe levels ($r = -0.453$) (Fig. 1). Similarly, a significant inverse correlation was noted between BLL and serum Ca ($r = -0.193$; $p<0.05$), serum Cu ($r = -0.303$; $p<0.01$) levels among group-A participants. Interestingly, group-A participant's serum Cu levels correlated positively with serum Fe ($r = 0.383$; $p<0.01$) and Ca ($r = 0.199$; $p<0.05$) levels, but no such correlation was observed among serum iron, zinc and calcium levels. Whereas, no correlations were noted between BLL and clinical parameters, trace element levels among group-B participants.

Fig 1: Blood lead level correlations with serum Fe and serum Creatinine levels.



The blood Pb levels correlated significantly ($p < 0.01$) positively with serum creatinine and inversely with serum Fe levels among Group-A individuals.

Table 3: Distribution of Blood Lead Levels (BLL) of Group-A participants into categories with percentage of subjects

S. No.	BLL categories (µg/dL)	No. of Subjects	BLLMean±SD (µg/dL)	Percentage of Subjects	Cumulative Percentage
1	<25[@]	3	20.3±2.23	2.7	2.7
2	25.01 - 30.00[§]	3	27.7±1.82	2.7	5.4
3	30.01 - 40.00[†]	1	36.1	0.9	6.3
4	40.01 - 50.00[‡]	3	45.6±0.49	2.7	9.0
5	50.01 - 60.00	6	56.4±1.73	5.5	14.5
6	60.01 - 70.00	10	65.7±2.73	9.1	23.6
7	70.01 - 80.00	15	75.7±3.13	13.6	37.2
8	80.01 - 90.00	18	85.7±3.43	16.4	53.6
9	90.01 - 100.00	15	96.2±2.73	13.6	67.2
10	>100.01	36	136.2±38.22	32.7	100

@ Cut-off levels (<25.0µg/dL) for work place exposure specified by CDC-NIOSH- ABLES;

§ Cut-off levels (<30.0µg/dL) for work place exposure specified by ACGIH-BEI;

† Cut-off levels (<40.0µg/dL) for work place exposure specified by OSHA; CDC; WHO.

‡ Cut-off levels (<50.0µg/dL) for work place exposure specified by United Kingdom Health and Safety Executive.

*Recommendations to Employer and Employee*s

According to industrial laws, the management has been practicing the relocation of the employees whose BLL were > 50 µg/dL to 'no-Pb exposure work' and an appropriate treatment by the physician. As part of current study, we have also suggested the employer to relocate the group-A employees, based on their BLL to 'no-Pb exposure work' at least in shifts, since 91% of screened employees had BLL >50 µg/dL, followed by regular checkups for BLL. Further, the BLLs >80 µg/dL were referred to undergo appropriate intervention decided by the physician. Similarly, the participants, who have BLLs above 100 µg/dL were kept under periodical observation by the physician along with therapeutic intervention to reduce the blood Pb levels. In addition , the employees were educated to practice personal hygiene like usage of masks to prevent the exposure to respirable dust and hand gloves to avoid the Pb absorption through skin. It was advised to minimize hand-to-mouth activity while working, followed by thorough hand wash at the end of the duty. Further, the employees were briefed about various adverse effects of chronic Pb exposure.

DISCUSSION

The incidence of battery industry Pb exposure is highest among all other occupational Pb exposures. In the current study, only 6% of the participants from group-A had BLL less than 30 µg/dL, the cut-off-limits specified for Biological Exposure Indices (BEI) by ACGIH (2012). In addition, 94% of group-A participants have BLL >40 µg/dL, the action limits specified by 'Occupational Safety and Health Administration' (OSHA), CDC and WHO (CDC-MMWR, 2014). One third participants from the battery industry had alarming blood Pb levels (>100 µg/dL) suggesting the persistent exposure to Pb at work place. This observation substantiates the "poor hygiene and inappropriate safety measures" that are being practiced at the small scale industrial sector in the developing nations. The recent report from India has also documented the elevated BLL due to work place exposure in automobile workers and battery industry employees (Dongre et al., 2010; Bhagwat et al., 2008). According to various agencies, it is mandatory to transfer the employees, who had the BLL above 40 µg/

dL (SHARP-1999), ≥60 µg/dL OSHA (CDC-MMWR, 2014) to non-Lead exposed jobs without loss of pay and benefits. In addition, the workers who are at higher risk need to undergo the necessary therapeutic intervention. On the other hand, the group-B participants BLL were within the specified cut-off limits, indicates 'probability of exposure to Pb' through industrialization, vehicular pollution, urbanization is absent in the remote rural areas.

It is well known fact that circulatory blood Pb maintains a dynamic equilibrium with various biological compartments thus affects the body Pb burden and exerts toxicity on various soft tissues such as bone marrow, kidney, brain etc. (Flora et al., 2006; Madipalli et al., 2007; Sakai 2000). Hamadouche et al., (2009), have reported that Pb exposed rats have shown significant increase in cholesterol levels while decrease in serum albumin, Ca, Mg and ascorbic acid levels. In the current study we have also noted the similar observation of 'significant positive correlation between BLL and serum creatinine and inverse correlation with serum albumin' in group-A individuals, implying of renal toxicity. However, in the present study we did not observe correlation between BLL and serum cholesterol levels, but still a higher level of cholesterol was noted in group-A subjects who were exposed to Pb than group-B. Accumulating evidences suggest that nutritional status interact with environmental toxicants and thus modulate the susceptibility to toxicity. In the current study, higher prevalence of trace element deficiencies was noted in group-B as compared to group-A urban individuals. This observation is line with 'National Health and Family Survey 3', India (2007), reported that urban population had better nutritional status as compared to rural Indians, due to food consumption pattern such as eating chicken, meat, fish, eggs, leafy vegetables and fruits are high in urban population than rural population. This could be one of the reasons for nutritional status variation among the remote rural group-B subjects and urban group-A individuals. Supplementation studies on humans and rat models have shown that trace elements such as Ca, Fe and Zn can modulate susceptibility to Pb toxicity (Ettinger et al., 2007; Jamieson et al.., 2006; Jain et al., 2005; Muwakkit et al., 2008). Lead affects bone development and mineralization, and also has an antagonistic activity in calcium metabolism (Ettinger et al., 2007). In the current study, blood Pb levels have shown a significant inverse correlation with serum Fe, Ca and Cu levels among group-A individuals but not in group-B. It is well known that at molecular level Pb competes with Ca on calcium binding sites and may subsequently alter protein function and calcium homeostatis (Ettinger et al., 2007). Another important notion is uptake of Pb at mucosal surface through 'Divalent metal ion transporter – 1 (DMT-1)', which competes with divalent metal ions such as Fe, Zn and Ca. Nonetheless, in the current study serum Zn and Mg levels have neither inverse nor positive correlation with increased BLL as reported by others (Jamieson et al.., 2006).

The above observations are further strengthened by significant positive correlations among trace elements such as serum Cu levels with serum Fe and Zn, suggesting of collective effort of trace elements might mitigate the toxicity caused by Pb. Though the serum albumin and total protein levels were significantly higher in group-A as compared to group-B, an inverse relationship was observed between serum albumin and elevated BLL of exposed individuals only, which has no explanation at the moment. In the current study, the serum elemental deficiency was minimal, but a persistence exposure to Pb at workplace might have led to increased BLL which intern might result in higher serum creatinine levels among the group-A participants. In contrary, majority of the earlier studies have carried out either in children or pregnant women, who had subclinical Pb toxicity through chronic low level exposure to Pb (Ettinger et al., 2007; Jain et al., 2005; Muwakkit et al., 2008; Reddy et al., 2011).

CONCLUSION

In conclusion participants from group-A had elevated blood Pb levels due to persistence exposure to Pb at work place. Further, the nutritional and trace element status may have limited effect on elevated blood Pb levels of 'chronic high level exposure'. The current study has certain limitations such as lack of information on Pb levels at work place, soil and water in the vicinity of battery industry; therefore it is need for a comprehensive study. Regular BLL screening is mandatory to reduce the risks associated with such occupational Pb exposure.

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Conflicts of interest & funding:

Declared none.

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