Serum Iron, Zinc and its relationship with Blood lead levels among lead exposed worker from lead battery plant

Bhavani Shankara Bagepally, Ravibabu Kalahasthi, Tapu Barman

INTRODUCTION

Lead is unique in that man-made sources contribute almost solely to exposure in the post-industrial era. Lead exposure occurs mainly through the respiratory and gastrointestinal tracts. Approximately 30-40 percent of inhaled lead is absorbed into the bloodstream. [1]. Elevated BLL was depends on absorption and retention of ingested Pb [2]. It has been speculated that Fe and Pb compete for absorption in the small intestine and in children with inadequate amounts of Fe consumption, lead absorption will be increased [3]. Wolf et al [4] showed that iron therapy cause decreased BLL in children with Fe deficiency. Pb and Zn compete for similar binding sites on a metallothionen protein in the intestine, which is reposposible for metal-transport. The dietary suppletion of Zn in Pb poisoned rats found decreased Pb concentration in tissue [5]. Serum Fe and Zn levels were not differed in Pb exposed subjects as compared to controls [6]. Reduction of BLL was noticed on the supplementation of Zn and Vitamin-C for 24 weeks [7].

Iron deficiency is an apparent risk for heavy-metal poisoning in children [8]. The mechanisms of Fe absorption are similar to other divalent metals such as Pb, Cd and Mn. The Fe deficiency causes excess absorption of neurotoxic metals namely Pb, Mn and Cd [9]. There are still disagreements reported in the literature regarding the association between Fe deficiency and BLL. Several studies reported that the prevalence of Fe deficiency associated with high BLL [10-12], however other studies were not reported this association [13-15]. It is observed that all these studies were conducted mainly among children and also in subjects with anaemic condition, with limited literature among non-anemic adult working population.

Further animal studies showed that Fe deficiency will augment lead absorption from the intestine and reported that Pb did increase liver Fe levels in all Fe-adequate rats. It is concluded that, compared with other tissues, the blood-brain barrier largely restricts Pb uptake by the brain and not related to the Fe status of the animal [16]. The Pb exposures disrupt the structure of blood- brain barrier (BBB) by increase of permeability and facilitate the accumulation of Pb. Iron supplementation appears protects the integrity of BBB against Pb insults [17]. Thus the relationship between BLL and serum Fe concentration among healthy non-anaemic subjects is not reported, as major cause of lead toxicity among adults is mainly due to occupational lead exposure. The present study was conceived to examine the relation between BLL and serum Fe & Zn levels among non-anaemic healthy workers from lead battery manufacturing plant.

ABSTRACT

Objective: Present study was conceived to examine the relationship between blood lead level (BLL) and serum Iron (Fe) and Zinc (Zn) in lead-exposed workers from lead Battery plant. Methods: The study design was cross-sectional. Ninety seven male workers involved in the lead battery manufacturing plant were considered as study subjects. BLL, serum Zn and Fe were estimated using Atomic Absorption Spectrophotometer. Haematological parameters were determined by using an Fx-19E haematology analyser. The renal function tests - creatinine and blood urea nitrogen (BUN) determined by standard methods. The statistical analysis was performed on SPSS version 16. Results: The body mass index (BMI) of subjects was 25.8±3.0. The systolic blood Pressure (SBP) was 127.8±14.5 mm Hg while diastolic blood Pressure (DBP) was 77.8±10.8 mm Hg. The workers with > 30 µg/dL BLL (Fe 1745 ± 723 µg/L) had significantly (P<0.05) lower serum Fe levels as compared to workers with ≤ 30 µg/dL BLL (Fe 2063±784 µg/L), serum Zn levels (0.70 ± 0.13 µg/ml versus 0.74 ± 0.14 µg/ml respectively) and renal function tests(serum creatinine and BUN) did not differ between these groups. The serum Fe showed significant negative correlation (r=-0.242, P=0.017) with BLL. Multivariate analysis between the four quartiles (Q1 to Q4) based on BLLs showed that the SBP (F= 3.483, with 95% CI of 125.2 to 130.6 mm of Hg, P=0.004) and mean arterial pressure (MAP) (F= 2.505 with 95 % CI of 92.4 to 96.6 mm of Hg, P=0.027) significantly differed between four BLL quartiles with trends of increase of SBP as well as MAP with higher BLL. Conclusion: Study observed negative association between BLL and serum Fe among the non-anaemic lead battery workers and reiterated the earlier finding of positive association between blood pressure and BLL.

KEY WORDS: Blood lead; Serum Iron and Zinc; Blood pressure and haematological
METHODS

The study involved a total of ninety seven male workers from a lead battery manufacturing plant located in Tamilnadu, India. The workers were randomly selected from the cohort of workers from battery manufacturing plant with pre-tested haemoglobin level of ≥13gm/dL. Informed written consent was obtained from each of the subject. The institutional ethical committee approved the study. The demographic details such as age, experience, occupational history, smoking, and alcohol consumption habits among workers were collected using a pre-designed validated questionnaire through personal interview. Body mass index (BMI) was calculated by noting the height and weight of each worker and expressed as kg/m². The blood pressure was measured on right arm, while the worker was in a sitting position using a standard mercury sphygmomanometer and mean arterial blood pressure (MAP) was calculated as sum of diastolic blood pressure (DBP) and one third of pulse pressure (difference between systolic blood pressure and diastolic blood pressure). Hypertension classification was performed using 7th Joint National Committee recommendation [18].

Blood sample collection

From each subject five ml (1 ml in K3-EDTA + 2 ml in heparin tubes + 2 ml in plain tubes) of whole blood were collected. 2 ml of heparinised blood sample was used for determination of BLL. Two ml of whole blood sample was collected in plain tubes, centrifuged at 3000 rotations per minute for 10 min at 4°C for separation of serum and red blood cells (RBC). The serum samples were used for assessment of trace elements such as iron (Fe) and Zinc (Zn). The whole blood sample with K3-EDTA (Tri-potassium- Ethylene diamine tetra acetic acid) was used to estimation of haematological parameters.

Haematological parameters

Red blood cell count, haemoglobin (Hb%), hematocrit (Hct), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) were determined by using a Fx-19E automated haematology analyser.

Renal function tests

Serum creatinine and blood urea nitrogen in study subjects were estimated by using prietest diagnostic kits and clinical chemistry analyzer, and expressed as mg/dL.

Estimation of blood lead

Two ml of venous whole blood was collected in a heparinized vacuette from the study subjects and was stored at -20°C until the analysis. Using ETHOS-D, (Milestone Microwave Laboratory Systems, Italy), 2 ml of whole blood was digested with 2 ml of nitric acid (HNO₃) and 0.2 ml of hydrogen peroxide (H₂O₂) 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 Atomic Absorption Spectrophotometer (GBC Avanta P, Australia). A standard solution of 20 µg/dL of Pb was prepared from the stock standard solution obtained from the Merck (1.19776.0500) and added to the lowest concentration of the sample. The analysis found 100% recovery with % RSD at <0.5 for three replicates and the BLL was expressed as µg/dL.

Trace elements estimation

Fe and Zn were determined by Flame Atomic Absorption Spectrometry (AAS) employing a GBC-Avanta P with deuterium background correction. The elemental hollow cathode lamp of Iron λ 248.3 and Zinc λ 213.9 nm (GBC Australia), air-acetylene flame and double distilled water as diluents were used in this estimation. All measurements were performed using integrated absorbance mode.

Statistical Analysis

Software package SPSS, version 16.0 for windows was used for the analysis of data. The descriptive statistics was used to analyze the data and continuous variables were presented as mean ± standard deviation. For comparison of continuous variables between blood lead levels ≤30 µg/dL & >30 µg/dL independent sample t test was used. To examine the relationship between the BLLs with Fe & Zn levels along with haematological parameters. The subjects were subgrouped in four quartiles based on the BLLs distribution and compared using multivariate analysis of covariance while statistically controlling the effects of age, years of experience and BMI. The parametric Pearson’s correlation test was used to examine the relationship between the BLL along with other continuous variables. The probability value < 0.05 considered as significant.

RESULTS

All 97 workers were males, aged about 35.5 ± 5.1 years, with working experience in the lead battery manufacturing plant for about 12.4 ± 3.9 years (range 3 to 22 years). The BMI of subjects was 25.8 ± 3.0. The systolic BP was 127.8 ± 14.5 mm of Hg while diastolic BP was 77.8 ± 10.8 mm of Hg. The serum creatinine level was 1.04 ± 0.20, Blood urea nitrogen 12.23 ± 2.40 mg/dL with haemoglobin of 14.6 ± 1.197 gm/dL. The blood pressure was measured on right arm, while the worker was in a sitting position using a standard mercury sphygmomanometer and mean arterial blood pressure (MAP) was calculated as sum of diastolic blood pressure (DBP) and one third of pulse pressure (difference between systolic blood pressure and diastolic blood pressure). Hypertension classification was performed using 7th Joint National Committee recommendation [18].
>30µg/dL BLL (Fe 1745 ± 723 µg/L) had significantly (P=0.04) lower serum Fe levels as compared to workers with ≤30µg/dL BLL (Fe 2063 ± 784 µg/L), however serum Zn levels did not differ between the groups as tabulated in Table 1.

Pearson’s Correlation test was used to examine relation between BLL and serum Fe showed significant negative correlation (r=-0.242, P=0.017) that persisted even after adjusting for age, experience and BMI (Figure A). Significant inverse association was noted between BLL and serum Fe among normotensive (r= -0.399, P=0.002) and hypertensive workers (r= -0.25, P=0.016) (Figure-B).

Further a multivariate analysis was performed between the four quartiles (Q1 to Q4) of workers based on BLL with adjusting for the effects of age, experience and BMI. The results showed that the SBP (F=3.483, with 95% CI of 125.2 to 130.6 mm of Hg, P=0.004) and mean arterial pressure (MAP) (F= 2.505 with 95 % CI of 92.4 to 96.6 mm of Hg, P=0.027) significantly differed between four BLLs quartiles. The direction of difference was observed to be increasing in SBP & MAP with higher the BLL. The results are tabulated in Table 2.

Similarly on multivariate analysis between four quartiles of Fe, SBP (F=3.985, P=0.001), DBP (F= 2.55, P=0.058), MAP (F= 3.29, P=0.006), and also BLL (F=3.63, P=0.003) significantly differed between quartiles while correcting for the effects of age, experience and BMI. The direction of difference was observed was increase in blood pressure as well as BLL with decrease of Fe.

**Table 1.** Showing the comparison of parameters between workers with BLL ≤30 versus >30µg/dL.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>BLL ≤ 30 µg/dL (n=47)</th>
<th>BLL &gt;30 µg/dL (n=50)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body mass index(Kg/m²)</td>
<td>25.97 ± 3.25</td>
<td>25.63 ± 2.78</td>
</tr>
<tr>
<td>Experience (years)</td>
<td>13 ±3</td>
<td>11 ± 4 *</td>
</tr>
<tr>
<td>Creatinine (mg/dL)</td>
<td>1.03 ± 0.25</td>
<td>1.05 ± 0.16</td>
</tr>
<tr>
<td>Blood urea nitrogen(mg/dL)</td>
<td>11.81 ± 2.23</td>
<td>12.61 ± 2.52</td>
</tr>
<tr>
<td>RBC(Cell/cumm)</td>
<td>4.19 ± 0.35</td>
<td>4.24 ± 0.49</td>
</tr>
<tr>
<td>Hb(g/dL)</td>
<td>14.74 ± 1.12</td>
<td>14.53 ± 0.81</td>
</tr>
<tr>
<td>Hematocrit (%)</td>
<td>37.21 ± 2.59</td>
<td>37.06 ± 2.62</td>
</tr>
<tr>
<td>MCV(µL)</td>
<td>90.45 ± 4.81</td>
<td>88.89 ± 6.42</td>
</tr>
<tr>
<td>MCH(µL)</td>
<td>35.70 ± 2.36</td>
<td>34.80 ± 3.59</td>
</tr>
<tr>
<td>MCHC(µg/dL)</td>
<td>39.54 ± 1.65</td>
<td>38.46 ± 5.29</td>
</tr>
<tr>
<td>SBP (mm of Hg)</td>
<td>127.30 ±16.03</td>
<td>128.44 ±13.13</td>
</tr>
<tr>
<td>DBP (mm of Hg)</td>
<td>77.30 ±11.33</td>
<td>78.36 ±10.29</td>
</tr>
<tr>
<td>Blood pressure n (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(1) Normotensive</td>
<td>17(36)</td>
<td>16(32)</td>
</tr>
<tr>
<td>(2) Pre-hypertensive</td>
<td>20(43)</td>
<td>21(42)</td>
</tr>
<tr>
<td>(3)Stage-1 hypertensive</td>
<td>06(13)</td>
<td>12(24)</td>
</tr>
<tr>
<td>(4) Stage -2 hypertensive</td>
<td>04(08)</td>
<td>01(02)</td>
</tr>
<tr>
<td>MAP (mm of Hg)</td>
<td>93.96 ±11.89</td>
<td>95.05 ±10.29</td>
</tr>
<tr>
<td>Zinc (µg/ml)</td>
<td>0.70 ±0.13</td>
<td>0.74 ±0.14</td>
</tr>
<tr>
<td>Iron (µg/L)</td>
<td>1745 ± 723</td>
<td>1745 ± 723*</td>
</tr>
</tbody>
</table>

* P<0.05 (Student t-test), RBC-Red Blood Cells, Hb-Haemoglobin, MCV-mean Corpuscular Volume, MCH- Mean Corpuscular Haemoglobin, MCHC- Mean Corpuscular Haemoglobin Concentration, SBP-Systolic Blood Pressure, DBP-Diastolic Blood Pressure, MAP-Mean Arterial Pressure.

**Figure A** showing the scatter plots depicting the association between blood lead level and serum Fe with marginal box plots.(Dotted lines represents the 95 % CI, green line is fitted line and red line is least square line).  **Figure-B** showing the association between blood lead level and Serum Fe along with the hypertensive status of the workers.
DISCUSSIONS

Present study examined the relationships of BLL, serum 
Fe, and Zn among workers in non-anaemic lead battery 
manufacturing plant. The study observed inverse relation
between the BLL and serum Fe levels, and no significant
association was found between BLL and serum Zn, 
which persisted even after adjusting age, experience 
and BMI as confounders. Fe and Zn are essential elements
for the maintenance of life and health. Lead is not an
essential metal and also harmful to human health. Fe &
Zn deficiency was associated with elevated BLL [20-
23]. Among Pb exposed workers, low levels of serum Fe
was associated with haematological parameters including
anaemia and not due to Fe deficiency [10, 24]. During the
present study, we noted significantly decreased serum Fe
in non-anaemic workers. A negative association was noted
between BLL and serum Fe in non-occupational Pb exposed
male subjects and the findings, suggested a competition
between BL and Fe and its binding capacity proteins
and feedback inhibition by heme on the iron transport
through the erythrocyte precursor membrane [25]. Choi et
al [11] reported inverse association between serum Fe and
BLL. The present study also reported inverse association
between BLL and serum Fe. Among Pb exposed workers
unaffected serum Zn and Fe concentration were reported
and not specifically attempted in non anaemic subjects [6].
Another study also noted low serum Fe, haematological
parameters with high BLL among anaemic subjects [26].
Earlier studies have noted lower Zn and Fe levels among
subjects with high BLL, however at the same time majority
of these observations were from anaemic subjects, thus
limiting the interactive relation between the Pb with these
metals [10-12]. Present study included specifically non-
anaemic subjects and observed inverse relationship between
the serum Fe and BLL with no significant difference in
serum Zn concentration.

Further the present study observed association in terms
of increase in BLL as well as decrease in Fe levels with
the higher BP recordings. Minerals intake was negatively
associated with blood pressure [27]. In the present study,
we noted negative association between serum Fe and blood
pressure. Fe deficiency causes cardiovascular morbidities
through hypertension [28]. The risk of coronary artery
diseases was associated with low levels of serum Fe and high
BLL [29]. A negative association was noted between serum
Zn and DBP [30]. Adequate mineral supplementation
reduces the blood pressure [31]. Tanya and Mandal [32]
reported a positive association between serum Zn and
blood pressure (DBP and SBP). Among artisans exposed Pb
had decreased plasma Zn with increase BLL [33]. However
present study, found no significant association between
serum Zn and blood pressure readings of workers possibly
due to specific non-anaemic subjects with normal serum
Zn concentration.

Human exposure to Pb occurs primarily through diet,
air, drinking water and ingestion of paint chips where
absorption increases mainly in persons suffering from Fe
and Ca deficiency [34]. Many studies especially in paediatric
group have revealed that Fe deficiency can elevate BLL
with increased absorption in gastrointestinal tract [13, 35-
37]. The hypothesis for this increase in lead absorption is,
recent research have shown an iron transport channel in the
intestine named the divalent metal transporter-1 (DMT1),
which is regulated by iron regulatory proteins 1 and 2 [38-
Bagepally, et al.: Blood lead and trace elements in lead battery work

9. The finding of positive association between elevated blood lead levels (BLL) and serum Fe levels even among the non-anaemic subjects.

In conclusion, this study observed negative association between BLL with the serum Fe even among non-anaemic subjects.

Our results showed that a higher the BLL is associated with an increase of systolic blood pressure and mean arterial pressure. These positive associations were reported in the earlier literature [43-44]. Even the animal studies have showed that low-dose of chronic lead exposure increases systolic arterial pressure, myocardial contractility [45] and vascular reactivity of rat aortas [46]. These findings of association between increased BLL and elevated blood pressure is also reported in African Americans population [47].

Some of the limitations of the study were no detailed diet history was taken from subjects however; none of study subjects were on any dietary supplementation. Due to cross sectional nature long term relations between Fe and Zn with Pb can’t be recognized as well as complex relationships/interactions between Pb and other micronutrients was not assessed. Study includes the interaction between Fe, Zn and BLL has been reported among non anaemic lead battery workers from India. Study also reported relationship between Serum Fe and BLL with blood pressure (SBP & MAP).

In conclusion, this study observed negative association between the BLL and serum Fe levels even among the non-anaemic lead battery workers and reiterated the earlier finding of positive association between elevated blood pressure and higher blood lead levels.

REFERENCES

19. American Conference of Governmental Industrial Hygienists: The documentation of threshold limit values and Biological Exposure Indices of chemical and physical agents. Cincinnati, USA. 2011.