Occupational lead exposure and liver functions in battery manufacture workers around Kolhapur (Maharashtra)

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Abstract: The magnitude of occupational lead exposure and its impact on liver functions was assessed in battery manufacture workers around Kolhapur (Maharashtra). Blood lead, urine lead, erythrocyte zinc protoporphyrin, urine δ-amino laevulinic acid and porphobilinogen levels were measured together with standard routine biochemical tests of liver functions in battery manufacture workers along with non-exposed controls subjects. Blood lead levels were considerably high in the workers (53.63 ± 16.98; range 25.8 – 78 μg/dl) compared to the controls (12.52 ± 4.08; range 2.8 - 22 μg/dl). The liver functions in these workers were normal, demonstrating preservation of hepatocellular and synthetic functions of liver. There was good correlation (r = 0.67) between blood lead and urinary δ-amino laevulinic acid levels in the worker group and no such correlation was observed in case of the control group (r = 0.02). The results indicates that early screening and regular monitoring of industrial workers by urinary δ-amino laevulinic acid levels is urgently needed to prevent long term adverse effects of lead exposure.

Key words: Lead, Occupational exposure, Liver functions, Battery recycling

Introduction

Lead is a ubiquitous and versatile metal, which has been used by humankind for over 6000 years and is today one of the most widely distributed toxin in environment. The source of lead in our environment may be natural or anthropogenic. The versatile properties of lead are utilized for variety of industrial applications including battery manufacturing [1]. In many countries of the world, occupational exposure to lead that results in poisoning, both moderate and clinically symptomatic, is still common [2-4]. Occupational exposure is entirely unregulated in many developing countries and little monitoring is conducted in developed countries [3-5]. Battery manufacturing and recycling is usually manual process in small scale industrial units. Poor hygiene and inappropriate protection increase the risk of lead poisoning in factory workers. Apart from the strong effects on haematopoetic system, lead is known to affect other systems including liver [1]. There are confusing reports on the effects of lead on liver functions [3, 4]. The present study attempts to assess the magnitude of lead exposure in an occupational setting and its impact on the liver function of battery manufacture workers (BMW) around Kolhapur in Maharashtra.
Material and methods

This is a case control study involving 30 employees from eight different small scale units engaged in battery recycling and manufacture of lead-acid batteries as the study groups. All the BMW subjects were in the range of 20-40 years of age. Non-Smokers, non-alcoholic subjects, who were engaged in battery manufacturing and recycling work for more than 6 hrs per day with duration of exposure from 2-20 yrs, were only selected for study. Age matched 35 normal healthy subjects living in rural areas since childhood around 30 kms from Kolhapur city served as non exposed controls (NEC). Socio-economic status of all subjects was average as assessed on oral interview. None of the subjects had a past history of major illness. Dietary intake and food habits of all subjects were similar as revealed by personal interview of the subjects. Subjects who were on drugs for minor illness were excluded from study to eliminate the effect(s) of drugs on the biochemical parameters being measured. Due to these stringent criteria the sample size was reduced to 30 only. Factory owners and workers were explained about study objectives and informed consent was obtained from all workers. Demographic, occupational and clinical data were collected by using questionnaire and interview. The entire experimental protocol was approved by institutional ethical committee and utmost care was taken during the experimental procedure according to the Helsinki Declaration of 1964 [6].

Random venous blood samples were collected from both the groups, 3-4 ml whole blood samples were collected in plain screw capped polypropylene tubes, coated with heparin while remaining 2 ml was collected in eppendorf polypropylene micro tubes containing EDTA. Random urine samples were also collected. Liver function tests (LFT) parameters included were serum total proteins (TP), albumin, alanine transaminase (ALT), aspartate transaminase (AST) and bilirubin, were measured in serum by using standard methods [7,8] on the same day of sample collection. The reagents were ready to use kits obtained from M/s Accurex Biomedical Pvt Ltd. Other biochemical parameters measured were blood lead (BPb) and urine lead (UPb) by atomic absorption spectrophotometer [9], erythrocyte zinc protoporphyrin (ZPP) by hematofluorimeter [10], urinary \( \delta \)-amino laevulinic acid (ALA) [11] and porphobilinogen (PBG) [12] were measured in small batches within a week after sample collection. Samples were stored at 2-8°C. Statistical analysis included unpaired, two tail students’t’ test between control and BMW groups. Pearson’s correlation equation was used to evaluate correlation between various parameters in the control and BMW groups separately.

Results

The observations and results are shown in tables. Most of the workers had major complaints of anorexia (40%), muscular pain (36.7%), headache (30%), abdominal (intestinal) colic and other neurological manifestations. Majority of workers had multiple signs and symptoms whereas controls did not show any clinical symptoms as described for the workers. The BPb and UPb levels were significantly high in BMW as compared to the controls. Minimum BPb level in BMW was 25.8  \( \mu \)g/dl
while maximum level in NEC was 22 µg/dl. The UPb levels were also similar to BPb (Table-1).

**Table 1: Biochemical parameters in the subjects**

<table>
<thead>
<tr>
<th>Parameter (units)</th>
<th>BMW n=30</th>
<th>NEC n =35</th>
<th>t value</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 BPb (µg/dl)</td>
<td>53.65 ± 15.53 (25.8 – 78.0)</td>
<td>12.59 ± 3.99 (2.8 – 22.0)</td>
<td>14.088*</td>
<td>3.53872 x 10^-15</td>
</tr>
<tr>
<td>2 UPb (µg/dl)</td>
<td>20.04 ± 14.31 (5.2 – 62.8)</td>
<td>6.97 ± 3.53 (1 – 13.2)</td>
<td>4.832*</td>
<td>3.88905 x 10^-05</td>
</tr>
<tr>
<td>3 ZPP (µg/dl)</td>
<td>135.9 ± 201.5 (10 – 972)</td>
<td>8.26 ± 7.46 (1 – 35)</td>
<td>3.4683*</td>
<td>0.007297</td>
</tr>
<tr>
<td>4 ALA (mg/dl)</td>
<td>38.15 ± 16.45 (9.3 – 74.9)</td>
<td>9.58 ± 5.27 (2.5 – 17.5)</td>
<td>9.120*</td>
<td>1.12802 x 10^-10</td>
</tr>
<tr>
<td>5 PBG (mg/dl)</td>
<td>14.58 ± 5.04 (7.0 - 24.7)</td>
<td>10.09 ± 2.78 (3.5 – 15.8)</td>
<td>4.3457*</td>
<td>8.23511 x 10^-05</td>
</tr>
</tbody>
</table>

Values are mean ± SD. Those in parenthesis are range of values. BMW = Battery Manufacture workers; NEC = Non exposed controls, BPb = Blood lead; UPb = Urine lead; ZPP = Erythrocyte zinc protoporphyrin; ALA = Urinary δ-Amino levulinic acid; PBG = Urinary porphobilinogen. *p< 0.001).

LFT parameters in the subjects are shown in Table-2. Serum proteins levels were marginally low, in BMW as compared to the NEC. Serum bilirubin levels were significantly higher as compared to NEC. AST and ALT levels were marginally higher in BMW than NEC. ZPP levels were significantly high in BMW as compared to the NEC. It is found that urinary ALA and PBG levels were significantly higher in BMW as compared to NEC.
### Table 2: Liver Function Tests parameters in BMW

<table>
<thead>
<tr>
<th>Parameter (Units)</th>
<th>BMW (n=30)</th>
<th>NEC (n=35)</th>
<th>t value</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 Bilirubin (mg/dl)</td>
<td>1.06 ± 0.41 (0.53 – 2.0)</td>
<td>0.76 ± 0.31 (0.4 – 1.4)</td>
<td>3.283**</td>
<td>0.003274</td>
</tr>
<tr>
<td>2 Total Protein (gm/dl)</td>
<td>7.70 ± 0.51 (6.7 – 8.4)</td>
<td>7.99 ± 0.49 (6.8 – 8.8)</td>
<td>2.327**</td>
<td>0.02342</td>
</tr>
<tr>
<td>3 Albumin (gm/dl)</td>
<td>4.39 ± 0.19 (4.35– 4.93)</td>
<td>4.60 ± 0.14 (4.0– 4.73)</td>
<td>5.000</td>
<td>2.96723 x 10⁻⁶⁶</td>
</tr>
<tr>
<td>4 Globulins (gm/dl)</td>
<td>3.10 ± 0.50 (2.0 - 4.45)</td>
<td>3.61 ± 0.43 (2.7 - 4.5)</td>
<td>4.371</td>
<td>6.20737 x 10⁻⁶⁵</td>
</tr>
<tr>
<td>5 A:G quotient</td>
<td>1.53 ± 0.35 (1.0 - 2.69)</td>
<td>1.22 ± 0.17 (0.92-1.7)</td>
<td>4.424</td>
<td>8.16177 x 10⁻⁶⁵</td>
</tr>
<tr>
<td>6 AST (U/L)</td>
<td>31.94 ± 8.01 (15 - 51)</td>
<td>28.66 ± 7.02 (11– 38)</td>
<td>1.744*</td>
<td>0.086799</td>
</tr>
<tr>
<td>7 ALT (U/L)</td>
<td>17.77 ± 7.25 (4 – 26)</td>
<td>16.02 ± 8.62 (5– 35)</td>
<td>0.889*</td>
<td>0.378125</td>
</tr>
</tbody>
</table>

Values are mean ± SD. Those in parenthesis are range of values, BMW = Battery Manufacture workers; NEC = Non exposed controls; AST = Aspartate transaminase; ALT = Alanine transaminase; **P< 0.005 , *p<0.05

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**Fig.1:** Relationship between blood lead levels (BPb range 25.8 – 78.5 µg/dl) and δ-aminolaevulinic acid in urine (ALA) of lead exposed BMW; n = 30, Correlation coefficient (r) = 0.66, p < 0.001; y = 0.687x + 1.0493.
There was good correlation between BPb and ALA values in BMW (r = 0.6723), (Fig 1) and there was no such correlation observed in case of the control group (r = 0.02), (Fig 2).

In other words, ALA values increased parallel with BPb values or the ALA values reflected BPb values. However, there was no satisfactory correlation between BPb and PBG values (r = 0.119) in this study.

**Discussion**

Battery recycling is an important source of exposure to inorganic lead. Battery recycling and manufacturing involves use of metallic lead for making grids, bearing and solder. Manufacturing process is usually manual and involves release of lead vapors, particles, debris and the lead oxides that cause considerable environmental pollution and severe lead poisoning. BPb in BMW was much higher than the NEC and correlated with the degree of exposure. The range of lead in blood of BMW was wider (25.8 – 78 µg/dl) than in NEC (2.8 - 22 µg/dl). Median levels were 53.45 in BMW and 13.0 in NEC. BPb levels depend on the equilibrium between absorption, storage and excretion [13]. Absorption of lead ordinarily results in rapid urinary excretion. This is clearly observed from the values of UPb in the subjects. It is accepted now that BPb levels generally reflect acute (recent/current) exposure and it
is also influenced by previous storage [13]. The range of BPb level reflected the duration of exposure in BMW when compared to the NEC. Such high BPb levels are expected to produce clinical symptoms of lead intoxication. These were observed in BMW subjects. Most common symptoms observed were anorexia, muscular pain and headache (Table-I).

Serum bilirubin was slightly higher in BMW as compared to NEC. Elevation of serum bilirubin is reported in several studies [14]. High concentration of lead has been shown to produce morphological changes and destruction of red cells when administered in vitro and in vivo [15]. Therefore, it appears that the higher bilirubin level could be a consequence of intravascular hemolysis, albeit in milder degree due to elevated BPb level in BMW.

Serum proteins are a gross measure of protein status and it reflects major changes in liver functions. The differences in serum protein levels are noticeable in BMW. The mean values of serum proteins were lower in BMW as compared to NEC. These are still within the reference interval specified for the respective methods. However, there was greater variation in globulins. Hence the mean as well as SD values for A:G quotient was higher in BMW. Further, the changes seen in serum proteins are clinically not significant. Similar results on serum proteins in automobile workshop workers are reported [4] but not by others [3]. Therefore it is reasonably said that the synthetic functions of liver is not affected in the range of blood lead level seen in BMW (26 – 78 µg/dl). The hepatocellular serum enzymes (AST & ALT) levels in BMW were slightly higher than those in NEC. The range of values was within acceptable limits for assay methods used. These results are similar to those reported earlier [3, 4] except alkaline phosphatase which is reported to be low in occupational lead exposure [3]. These results suggest that hepatocellular / parenchymal function is conserved in BMW at the observed BPb range (highest at 78 µg/dl).

Lead is known to inhibit the activities of three enzymes in heme biosynthesis viz δ-aminolaevulinic acid dehydratase, coproporphyrin oxidase and ferrochelatase. Therefore, the formation and excretion of heme precursors such as ALA, PBG, coproporphyrin and ZPP is increases by lead exposure [16-18]. Lead inhibits ferrochelatase enzyme that incorporates iron into protoporphyrin-IX to form heme [16-18]. Decreased conversion of protoporphyrin builds up erythrocyte level, which then binds with zinc to form ZPP [18]. Mean ZPP levels were significantly higher in BMW as compared to NEC. Elevated values of blood ZPP are reported in automobile workers (4) with a range of 10.7 – 17.8 µg/dl, BPb and 27.4 – 39.2 µg/dl ZPP which is much lower than the present study (Table 1), indicating low level of exposure in automobile workers.

ZPP is not a suitable marker for screening occupational lead exposure because it is also reported to be elevated in chronic iron deficiency, hemolytic anemia, erythropoietic protoporphyria and chronic febrile illness [19]. Hence ZPP has low specificity in screening lead exposure. Several studies reported the limited usefulness of ZPP as a biological indicator of lead exposure when BPb levels are lower than 25 µg/dl [18-21]. Thus it appears that ZPP levels have poor utility in screening and confirming occupational lead exposure. Urinary ALA appears to be a better indicator.
for screening occupational lead exposure. ALA is also a convenient indicator for screening as it is non-invasive investigation in addition to sensitivity as compared to PBG and better specificity than ZPP.

The observations of this study indicate that occupational lead exposure still remains a concern in developing countries like India. Chronic lead exposure in unregulated small scale industrial units together with low economic conditions has cumulative effects on deteriorating health of BMWs. There is urgent need to monitor lead exposure using simple biological markers such as ALA in workers involved in lead battery manufacturing, recycling and similar other occupations where risk of lead exposure is high. Elevated random urinary ALA with a history of occupational lead exposure indicates greater risk of impending lead toxicity. Use of adequate protective and preventive measures together personal hygiene and better nutrition will certainly reduce long term adverse effects of lead in industrial workers.

Conclusion

BPb levels were considerably high with a range of 25.8 – 78 μg/dl in workers involved in manufacturing and recycling of lead batteries around Kolhapur (Maharashtra). The degree of elevation in BPb roughly paralleled with the duration of occupational lead exposure. The liver functions in these workers were not affected at the observed BPb range (highest at 78 μg/dl) as evident from the biochemical tests done. Urine ALA is most suitable and convenient marker to screen and confirm occupational lead exposure. Apart from saving costs on blood tests to detect lead toxicity, elevated urine ALA levels with a history of occupational exposure strongly indicates greater risk of impending lead toxicity. Early screening and regular monitoring of industrial workers is urgently needed to reduce long term adverse effects of lead exposure.

References

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