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Biochemical effects of lead exposure and toxicity on battery manufacturing workers of Western Maharashtra (India): with respect to liver and kidney function tests

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Abstract: *Background:* The battery recycling and manufacturing involves the use of metallic lead for making grids, bearing and solder. The process results in release of lead particles and lead oxide causing environmental pollution and severe lead poisoning. *Aims and Objectives:* To know the present scenario of the blood lead level and its biochemical effects on occupational lead-exposed population, mainly battery manufacturing workers in Western Maharashtra (India) with respect to liver and kidney functions tests. *Results:* The biochemical parameters such as Blood Lead (PbB) level ($p<0.001$, 542%), Alanine Transaminase (ALT) ($p<0.05$, 20.47 %), serum total bilirubin ($p<0.01$, 46.42%), and direct bilirubin ($p<0.001$, 90.9%), serum alkaline phosphatase ($p<0.001$, 33.38%), blood urea level ($p<0.001$, 25.85%) and serum creatinine ($p<0.001$, 22%) levels were significantly increased in battery manufacturing workers as compared to control subjects. Slightly decreased level of serum total proteins ($P<0.01$, 4.32 %), albumin ($P<0.01$, 4.81 %) and blood sugar ($p<0.001$, 13.49%) and no statistically significant alteration were observed in Aspartate Transaminase (AST), serum globulins, albumin/globulin ratio, indirect bilirubin, uric acid and cholesterol in the study group as compared to control group. *Conclusions:* This study revealed increased blood lead level in battery manufacturing workers of Western Maharashtra (India), indicating more absorption of lead, despite modern technical supports, considerable lead induced health hazards still exist. Alteration of Liver and kidney function tests though they are statistically significant, but are within the normal range, indicates that there was no evidence of severe disturbance of liver and kidney functions in battery manufacturing workers.

Keywords: Blood Lead, Liver and Kidney Function Tests, Battery Manufacturing.

Introduction

Lead is a heavy, low melting, bluish-gray metal that occurs naturally in the earth's crust. However, it is rarely found naturally as a metal. It is usually found combined with two or more other elements to form lead compounds [1]. As it is one of the first discovered and most widely used metal in human history and is, therefore, one of the metal most commonly encountered in the environment [2].

Its continued release into the environment as an exhaust emission product, as well as its widespread industrial use, has made lead a serious threat to human health [3]. Most lead used by industry comes from mined ores ("Primary") or from recycled scrap metal or batteries ("Secondary"). However, most lead today is "secondary" lead, obtained from lead-acid batteries. It is reported that 97% of these batteries are recycled [1].

The largest industrial use of lead today is for the production of lead batteries, extensively used in automobile industries. Other uses of lead include the production of lead alloys, use in soldering materials, shielding for X-ray machines, and in the manufacture of corrosion and acid resistant materials used in the building industry [4]. Lead has long been known to alter the hematological system by inhibiting the activities of several enzymes involved in heme biosynthesis, particularly δ-aminolevulinic acid dehydratase (δ -ALAD). Inhibition of δ -ALAD activity occurs over a

wide range of Pb in blood beginning at <10 µg/dL [1]. The anemia induced by lead is primarily the results of both inhibition of heme biosynthesis and shortening of erythrocyte life span, but lead can also induce inappropriate production of erythropoietin leading to inadequate maturation of red cell progenitors, which can contribute to anemia. Lead absorbed by the GIT comes from the intake of the lead in food, beverages and soil or dust in case of older children and adults and in occupational exposure population mostly from atmospheric air [5].

After absorption lead enters into the blood. Blood lead concentrations are currently regarded as the most reliable index of exposure to lead. Over 95% of blood lead is bound to the erythrocytes and seems to be in dynamic equilibrium with plasma lead [6]. Once absorbed it is distributed particularly to the liver and kidneys, and is then stored in the bones and cause damage to the organs including the liver, kidneys, heart and male gonads as well as causes effects to the immune system [1].

Lead interferes with the function of enzymes and essential cations (particularly calcium) in cells throughout the body [1, 7], and lead poisoning is usually associated with multi-systemic signs and symptoms [8-9]. The metal causes proximal renal tubular damage, characterized by generalized aminoaciduria, hypophosphatemia, with relative hyperphosphaturia and glycosuria accompanied by nuclear inclusion bodies, mitochondrial changes, and cytomegaly of the proximal tubular epithelial cells [1, 5]. Lead also affects normal liver functions, impairs the detoxification of xenobiotics (environmental toxins and drugs), alters tryptophan metabolism, and elevates serotonin and hydroxy indole acetic acid in brain, resulting in disturbed neurotransmitter functions [1, 5].

The high blood Lead level affects almost all organs and systems and impairs the normal functions of the body. Though it is well documented in literature, it is essential to know the present scenario of blood lead level and its effects on lead exposed population. Therefore, the aim of this study is to create lead awareness in the society and to see the blood lead level and its biochemical effects on occupational lead-exposed population mainly battery manufacturing workers in Western Maharashtra (India) with respect to liver and kidney functions tests.

Material and Methods

For this study total 82 subjects were included from Western Maharashtra, India, among whom 45 were battery manufacturing workers (study group) and 37 were normal healthy subjects (control group). The subjects of study group were aged between 20 to 40 years and similar age-matched normal healthy control subjects were taken from same area. Before data and biological specimen collection, both study and control group subjects were informed about the study objectives and health hazards of lead exposure and its toxicity. Written consent was obtained from subjects of both groups. Demographic, occupational and clinical data were collected by using questionnaire and interview.

Majority of battery manufacturing workers had major complaints of loss of appetite, intermittent abdominal pain, nausea, diarrhea, constipation and muscle pain. The socioeconomic status of all subjects of both groups was average. No subject had a history of any major illness. Dietary intake and food habits of all subjects were normal. Subjects who were taking drugs for minor illnesses, non-smokers, non-alcoholic healthy males were excluded from this study. The entire experimental protocol was approved by the institutional ethical committee, and utmost care was taken during the experimental procedure according to the Helsinki declaration of 1964 [10]. Blood samples were collected by puncturing the anticubital vein into evacuated tubes containing EDTA solution as anticoagulant.

Liver and kidney functions tests were measured using a fully automated biochemistry analyzer. The transaminases SGOT (AST) and SGPT (ALT) were measured by the UV-kinetic method [11] using reagents from M/S Accurex Biomedical Ltd. The conversion of NADH to NAD in both transaminase reactions was measured at 340 nm, as the rate of decrease in absorbance. Serum total proteins were measured by the Biuret method [12] using an M/S Accurex Biomedical Kit. Serum proteins react with

cupric ion in alkaline pH to produce a colored complex. The intensity of the color complex was measured at 546 nm and directly proportional to the protein concentration in the specimen. Serum albumin was measured by the BCG method [13] using reagents from M/S Beacon Ltd. Serum albumin binds with 3,3′,5,5′-tetra bromocresol sulfonapthalein (BCG) in acidic medium at pH 4.2, and the blue-green colored complex formed is measured at 600 nm. Serum globulins and the A/G ratio were calculated by using serum total proteins and albumin values.

Serum total bilirubin was estimated by the Jendrassik method [14] using an M/S Accurex biomedical kit. Serum bilirubin reacts with diazotized sulfanilic acid to produce azobilirubin (pink color). Dimethyl sulphoxide (DMSO) catalyzes the formation of azobilirubin from free bilirubin. The intensity of the pink color is proportional to the bilirubin concentration, measured at 546 nm.

Serum alkaline phosphatase was measured by King Armstrong method using Span diagnostics kit [15]. Phenol is release by an enzymatic hydrolysis from disodium phenyl phosphate under defined conditions of time, temperature and pH. This reacts with 4-aminoantipyrine in the presence of alkaline oxidizing agent to give a red colored compound which is measured at 520 nm against a reagent blank. Color development is rapid and stable for at least one hour in bright light. Sodium hydroxide is added immediately after incubation to raise the pH and stop the reaction. Potassium ferricyanide is the oxidizing agent and sodium bicarbonate is added to provide the alkaline medium.

Blood urea was measured by GLDH method [16] using an M/S Agape Diagnostics Kit. Blood urea is decomposed by urease to form ammonia and carbon dioxide. Ammonia combines with 2-oxo-glutarate in presence of glutamate dehydrogenase and NADH to form L-glutamate and NAD. The rate of NAD formation was measured at 340 nm and was directly proportional to blood urea. Each molecule of urea hydrolyzed liberates two molecules of NAD^+. Serum creatinine was estimated by Jaffes method [17] using an M/S Accurex biomedical kit. Serum creatinine in alkaline medium reacts with picrate to produce an orange color that absorbs light at 492 nm. The rate of increase in absorbance is directly proportional to the concentration of creatinine in specimen.

Blood glucose was measured by the Trinder, Young et al. enzymatic method using Autozyme kit [18-19]. Glucose oxidase converts glucose to gluconic acid. Hydrogen peroxide formed in this reaction, in presence of peroxidase (POD), oxidatively couples with 4- aminoantipyrine/phenol to produce red quinoneimine dye. This dye has an absorbance maximum at 505 nm. The intensity of the color complex is directly proportional to the glucose in specimen.

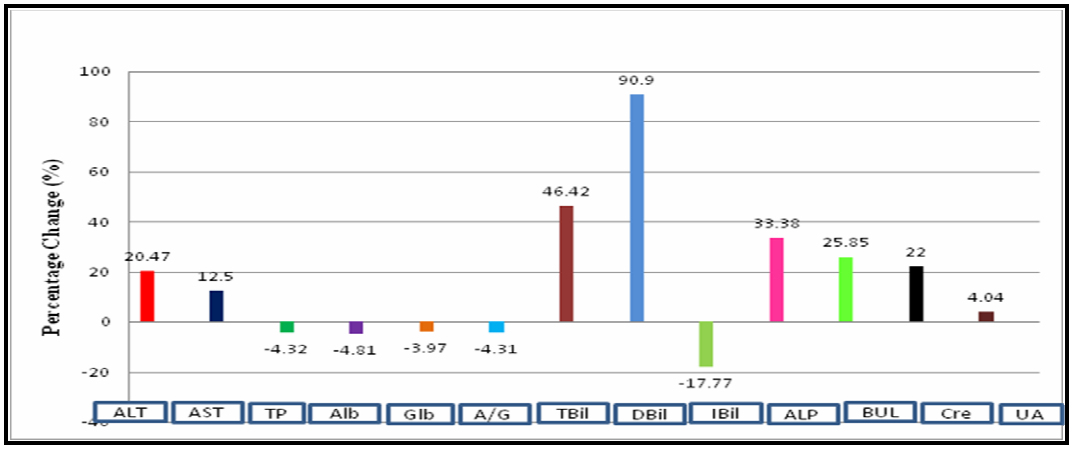
Serum cholesterol was measured by the Richmond, Tarbutton, and Gunter method [20].Cholesterol esterase hydrolyses cholesterol esters into free cholesterol and fatty acids. In the second reaction cholesterol oxidase converts cholesterol to cholest-4-en-3 one and hydrogen peroxide. In presence of peroxidase, H_2O_2 oxidatively couples with 4-amino-antipyrine and phenol to produce a red quinoneimine dye, having an absorbance maximum at 510 nm (500-530). The intensity of the red color is proportional to the amount of total cholesterol in the specimen.

Results

Table-1: Blood Lead Level and Liver Function Tests of Healthy Control Subjects and Battery Manufacturing Workers			
Sr. No.	**Biochemical Parameters**	**Control Group (N=37)**	**Battery Manufacturing Workers (N= 45)**
A	Blood Lead (µg/dl)	5.21± 3.27	33.50 ± 12.90***
B	**Liver Function Tests**		
1	ALT (Unit/ L)	28.87 ± 9.10	34.78 ± 12.32*
2	AST (Unit/ L)	30.40 ± 11.70	34.20 ± 11.80°
3	Serum Total Proteins (gm/dl)	8.10 ± 0.75	7.75 ± 0.45 **
4	Serum Albumin (gm/dl)	4.57 ± 0.35	4.35 ± 0.32**
5	Serum Globulin (gm/dl)	3.52 ± 0.77	3.38 ± 0.56°
6	Albumin / globulin Ratio	1.39 ± 0.45	1.33 ± 0.30°
7	Serum Total Bilirubin (mg/dl)	0.56 ± 0.22	0.82 ± 0.45**
8	Serum Direct Bilirubin (mg/dl)	0.22 ± 0.10	0.42 ± 0.25***
9	Serum Indirect Bilirubin (mg/dl)	0.45 ± 0.30	0.37 ± 0.23°
10	Serum Alkaline Phosphtase (KA Unit)	68.60 ± 23.50	91.50 ± 34.40***
Figures in table 1 indicate mean and SD. *p< 0.05, **p<0.01, ***p< 0.001, ° Non Significant as compared to control group. ALT- Alanine Transaminase, AST- Aspartate Transaminase			

Table-2: Blood Lead Level, Blood Sugar, Serum Cholesterol and Kidney Function Tests of Healthy Control Subjects and Battery Manufacturing Workers			
Sr. No.	**Biochemical Parameters**	**Control Group (N=38)**	**Battery Manufacturing Workers (N= 49)**
A	Blood Lead (µg/dl)	5.21± 3.27	33.50 ± 12.90***
B	Blood Sugar (mg/dl)	92.60 ± 10.90	80.10 ± 11.30***
C	Serum Cholesterol (mg/dl)	168. 40 ± 41.30	156.70 ± 39.40°
D	**Kidney Function Tests**		
1	Blood Urea (mg/dl)	19.84 ± 5.99	24.97 ± 6.09***
2	Serum Creatinine (mg/dl)	1.0 ± 0.18	1.22 ± 0.09***
3	Serum Uric Acid (mg/dl)	5.93 ± 1.69	6.17 ± 1.48°
Figures in table 2 indicate mean and SD. ***p< 0.001			

Fig-1: Percentage Change of Liver and Kidney Function Tests of Battery Manufacturing Workers with Respect to Control Subjects



ALT- Alanine Transaminase, **AST**- Aspartate Transaminase, **TP** - Total Proteins, **Alb**-Albumin, **Glb**-Globulin, **A/G**-Albumin /Globulin Ratio, **TBil**-Total Bilirubin, **DBil**-Direct Bilirubin, **IBil**-Indirect Bilirubin, **ALP**-Alkaline Phosphates, **BUL**-Blood Urea Level, **Cre**-Creatinine, **UA**-Uric Acid.

Discussion

A significant increase in blood lead (PbB) level ($p<0.001$, 542%) in battery manufacturing workers as compared to control subjects indicates the absorption of lead is still more in battery manufacturing workers inspite of all precautions taken to reduce the lead exposure and its toxicity. Well established battery manufacturing industry owners have provided cool air spray to the all workers, at the place where they stand during working hours for prevention of lead dust. A special lead free shampoo is given to all workers for hand wash and bathing. All workers regularly take bath immediately after work and use special full apron and mouth mask during working time. In most of battery manufacturing industries, all lead dust is collected and discarded in air by chimneys to reduce the lead exposure. Normally lead accumulates in bones followed by soft tissues in excessive lead exposure. Blood lead levels depend on the equilibrium between absorption, storage and excretion [21].

Battery recycling and manufacturing involves the use of metallic lead for making grids, bearing and solder. Manufacturing process is usually manual and involves the release of lead particles and lead oxide that may cause environmental pollution and severe lead poisoning. Poor hygiene and inappropriate protection might be the reason for increased blood lead level in battery manufacturing workers and also our result is consistent with other report in the literature [8-9, 22-25].

In this study the Alanine Transaminase (ALT) level is significantly increased ($p<0.05$, 20.47 %), but the alteration in Aspartate Transaminase (AST) level is not statistically significant in battery manufacturing workers as compared to control subjects. Slightly increased ALT level may be due to increased blood lead level in BMW and recently Similar results are also reported in the literature [26-27]. Slightly decreased levels of serum total proteins ($P<0.01$, 4.32 %) and albumin ($P<0.01$, 4.81 %) and no statistically significant alteration of serum globulin, albumin /globulin ratio were observed in battery manufacturing workers as compared to control. Decreased serum total proteins and albumin may be due to the high blood lead level in BMW. The increased blood lead level decreases the synthesis of albumin and other proteins reported in several earlier studies [24-25]. Also several studies have reported decreased proteins synthesis at high PbB level in experimental animals on lead exposure. The globin synthesis is inhibited by lead in rat bone marrow cells at concentrations as low as 1μmol/L [28]. Therefore, the estimation of total proteins is also valuable to detect the impairments of liver function in high lead exposure workers.

The serum total bilirubin level ($p<0.01$, 46.42%), serum direct bilirubin ($p<0.001$, 90.9%) were significantly increased, but serum indirect bilirubin level was not significantly altered in battery manufacture workers as compared to the control subjects. Increased serum total and direct bilirubin level may be due to the high blood lead level of BMW and these results are consistent with earlier reports in the literature. In several studies, it has been reported that the serum bilirubin level is significantly increased in lead poisoning cases [29-32]. Earlier Maugeri (1940) studied the industrial lead poisoning and found a increased serum bilirubin and increased excretion of stercobilinogen & urobilinogen and they have concluded that the lead anaemia was haemolytic type [33].

This report also gives the support to the theory that lead causes haemolysis. The jaundice and raised serum bilirubin was also reported in several cases of lead poisoning [34-35]. Cantarow and Trumper (1944) found that levels ranging from 1.0 to 2.3 mg/dl were not uncommon in lead poisoning cases and raised levels have also been reported by several studies [29-36]. The large concentrations of lead have been shown to produce morphological changes and destruction of red cells when administered in vitro or vivo. At concentrations of 0.15 M Pb haemolysis was observed microscopically in the spherical and hexagonal crenate forms produced although smaller crenate forms did not haemolyse [37]. This study demonstrates that the rate of haemolysis is more at high PbB level. Hence the serum bilirubin level is increased in battery manufacture workers as compared to control group.

Significant increase in serum alkaline phosphatase level ($p<0.001$, 33.38%) in battery manufacturing workers as compared to control subject may be due to increased blood lead. Similar increased in serum alkaline phosphatase level of entire family manufacturing lead acid batteries was reported by National Referral Centre for Lead Poisoning in India [38]. The blood urea level ($p<0.001$, 25.85%), serum creatinine ($p<0.001$, 22%) were significantly increased and serum uric acid was not altered significantly in battery manufacture workers as compared to the control group. Slight increased blood urea and serum creatinine may be due to high blood lead level in BMW. Whereas, significantly increased uric acid and no alteration of blood urea, serum creatinine were reported in Nigerian lead exposed workers [39].

Lead is known to cause proximal renal tubular damage, characterized by generalized aminoaciduria, hypophosphataemia, with relative hyperphosphaturia and glycosuria accompanied by nuclear inclusion bodies, mitochondrial changes and cytomegaly of the proximal tubular epithelial cells. Tubular effects are noted after relatively short-term exposures and are generally reversible, whereas sclerotic changes and interstitial fibrosis, resulting in decreased kidney function and possible renal failure, require chronic exposure to high lead levels. Increased risk from nephropathy was noted in workers with a PbB level of over 60 µg/dl [40].

Chronic nephropathy, which may progress to kidney failure, is the classic renal manifestation of lead toxicity. It appears to result from long-term and relatively high dose exposure of lead. The cell lining of the proximal tubules in the kidney are highly sensitive to lead. At PbB concentration below 25 µg/dl lead inhibit the metabolic activation of vitamin D, a transformation that occurs in these cells. Lead induces the formation of dense intranuclear inclusion bodies consisting of lead protein complex found at PbB concentration of 40-80 µg/dl. Hyperuricemic gout apparently resulting from increased re-absorption of uric acid by the tubular cells is a third metabolic complication of lead induced renal impairment [40]. The evolution of lead nephropathy is usually silent. The central events appear to be the progressive destruction of tubular cells and their replacement by fibrosis. Clinical manifestation of impairment, characterized by rise in blood urea nitrogen or serum creatinine, do not ordinarily become evident until 50-70 % of the nephrons are destroyed. Pathological late stage of lead nephropathy is characterised by interstitial fibrosis with atrophy and dilation of the tubules and relative sparing of glomeruli, in this stage intranuclear inclusion are infrequent [5].

The most important research need in the study of lead nephropathy is a reliable early biological indicator of the kidney damage induced by lead. Such a marker would permit better assessment of dose response relations and might enable determination of proportion of cases of renal failure caused by chronic exposure to lead a function which is presently unknown. Therefore, estimation of blood urea, uric acid and creatinine are not useful for screening the low lead exposure population.

Decreased blood sugar ($p<0.001$, 13.49%) and no alteration of serum cholesterol level were observed in battery manufacturing workers as compared to controls. However, in the study of battery workers of Lucknow city reported that the lead exposure increases cholesterol synthesis and transport to peripheral tissues whereas reverse cholesterol transport to the liver is not affected [40] and there was no significant correlation between blood lead and fasting blood sugar, triglycerides, HDL-Cholesterol, LDL-Cholesterol, total Cholesterol were reported [41].

Alteration of all these biochemical parameters in this study though statistically significant, but all are within the normal range and we have not observed any severe change in the biochemical parameters. Therefore, this study shows that there was no evidence of severe disturbance of liver and kidney functions in the occupational lead exposure such as in battery manufacturing workers may be due to low blood lead level as compared to earlier studies [24, 25]. The acute symptoms of lead poisoning including abdominal colic, constipation, anorexia, vomiting, headache, light-headedness, dizziness, forgetfulness, anxiety, depression, irritability, excessive sweating, muscle and joint pain were observed

in most of the BMW. Recognition of the early symptoms of lead exposure can minimize toxic effects, reduce neurological damage, and thus prevent permanent impairments among susceptible individuals exposed to levels that may have been considered safe [42]. The acute symptoms subside following cessation of exposure and the reduction of blood lead level. Repeated acute exposure or chronic exposure leads to more persistent neurological manifestations including peripheral neuropathy, muscle wasting and overt neuropsychological impairment. There is a need to protect the workers from the health hazards of occupational lead exposure. The potential risk of lead toxicity will persist unless safety measures are taken by the employers, social groups and government agencies. In addition, factories and workers should be included in a basic care plan to receive training on the proper use and application of personal protection devices and industrial hygiene measures.

Conclusions

This study revealed increased blood lead level in battery manufacturing workers of Western Maharashtra (India), indicating more absorption of lead despite modern technical supports considerable lead induced health hazards still exist. Alteration of liver and kidney function tests though they are statistically significant, but are within the normal range, indicates that there was no evidence of severe disturbance of liver and kidney functions in battery manufacturing workers. Frequent estimation of blood lead, liver and kidney function tests are very useful to see the absorption of lead and its effects on health of workers exposed lead. It is also useful for managers of battery manufacturing industries to shift the workers from areas of more exposure to those with lesser exposure.

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