Original Article

Clinical and Laboratory Findings of Lead Hepatotoxicity in the Workers of a Car Battery Manufacturing Factory

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ABSTRACT

Background: Occupational lead poisoning is common in workers of some industries, but lead hepatotoxicity has rarely been reported. Several animal studies have revealed lead induced liver damage but clinical studies concerning the manifestations of lead induced liver toxicity in humans are scares. This study was designed to investigate the clinical manifestations and pathological parameters of hepatic dysfunction and its relationship with blood and urine lead concentrations in a car battery-manufacturing workers.

Methods: This cross sectional study was carried out in Mashhad, Iran, during April-June 2011. One hundred and twelve workers underwent blood and urine sampling for determination of lead concentrations and liver function tests. Clinical signs and symptoms of possible lead hepatotoxicity were investigated.

Results: Mean (±SD) age of the workers was 28.78 (±5.17) yr with a daytime work of 8.67 (±1.41) h and mean work duration of 3.89 (±2.40) yr. Mean blood lead concentration (BLC) and urine lead concentration (ULC) were 398.95 (±177.41) µg/l and 83.67(±50) µg/l, respectively. We found no correlation between the clinical findings and BLC or ULC. A weak correlation (R: 0.27, *P*=0.087) between serum alkaline phosphatase concentration and BLC was obtained. No significant relationship was found between other liver function tests and BLC or ULC.

Conclusion: We found no specific clinical and laboratory abnormalities of liver in the workers of car battery manufacturer who had chronic lead toxicity. Further investigations with more specific laboratory tests such as LDH5 and gamma glutamyl transferase (GGT) as well as novel biomarkers of metal induced hepatotoxicity might be helpful in evaluating lead hepatotoxicity.

Keywords: Lead Poisoning, Liver, Liver Function Tests, Occupational Exposure, Transaminases.

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INTRODUCTION

Lead poisoning is considered a serious threat to human health, particularly to workers exposed to lead at their daily professions. This occupational disease may cause serious complications at some organs such as kidneys, brain, reproductive organs and liver [1, 2]. Occupational exposure to lead is common especially in developing countries where most industrial workers are not aware of the dangers involved.

Several animal investigations have been performed on hepatotoxicity of lead, although clinical studies concerning the manifestations of lead induced liver toxicity in humans are scares [3-6]. After exposure, most of the lead in blood bounds to red blood cells and the rest in plasma can distribute to several organs such as liver, kidney and brain. Absorbed lead accumulates mostly in soft tissues, bones and liver [7, 8]. Lead induces overproduction of reactive oxygen species, which can result in peroxidation damage to hepatic cell membranes. Damage to hepatic cells leads to release of transaminases and increases their levels in serum [9]. In high concentrations of lead cause erythrocyte

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hemolysis due to morphological changes and might cause increased levels of serum bilirubin [2]. There are contradiction as for alkaline phosphatase, whether it decreased or increased in lead poisoning [2, 10].

The present study was aimed at investigating clinical and pathological parameters of hepatic dysfunction and its relationship with blood and urine lead concentrations in workers of a car battery manufacturing factory located in east of Mashhad, Iran.

MATERIALS AND METHODS

This cross sectional study was performed between April and June 2011 on 138 male workers of a car battery-manufacturing factory (Niroogostaran) in Mashhad, Iran.

The research project was approved by the Medical Ethics Committee of the University of Mashhad. Before the study, all participants signed an informed consent form.

Demographic data and past medical histories were recorded in a predesigned validated questionnaire. None of participants had any history of hepatic, cardiac and kidney diseases before or during their job in the factory as well as no history of taking medications affecting liver function tests. Furthermore, none of them had taken chelating agents, such as meso-2, 3-dimercaptosuccinic acid (Succimer), 2, 3-dimercaptopropanol (Dimercaprol) or British anti lewisite (BAL), calcium disodium EDTA ($CaNa_2EDTA$) and D-penicillamine during the last 6 months. All participants were examined for signs of liver damage (included: icterus, tenderness of right upper quadrant, abdominal pain, nausea, vomiting, fatigue, acholic stool, urine darkness, pruritus, skin hyper pigmentation, anorexia, temporal muscle atrophy, hepatomegaly, splenomegaly, ascitis and palmar erythema) by an internist with sub-specialty in clinical toxicology (first author). Clinical signs and symptoms were recorded in a nominal YES/NO scale in a predesigned checklist.

From each worker, a sample (10 ml) of brachial venous blood was drawn; of which, 5 ml in heparinized tubes for analyzing blood lead concentration (BLC) and 5 ml in simple tubes to get serum for determination of liver function tests. Urine samples were also obtained from all participants. We used lead-free syringes and lead-free polyethylene containers to minimize the risk of lead contamination in the study.

Liver function tests included alanine and aspartate aminotransferase (ALT and AST), alkaline phosphatase (ALP), lactate dehydrogenase (LDH), bilirubin total and direct. Moreover, blood and urine lead concentrations were also evaluated. Biochemical parameters were determined in the Biochemistry Laboratory of Imam Reza Hospital of Mashhad by colorimetric method within four hours of sampling (Parsazmoon reagent™, Iran and BT 3000 biochemical autoanalyzer™, Italy). Hemolysed samples were excluded from the study. The normal ranges for these parameters were ALP (45 to 115 U/L), LDH (122 to 222 U/L), AST (8 to 48 U/L), ALT (7 to 55 U/L) and bilirubin (0.1 to 1.0 mg/dL).

Determination of Blood Lead Concentration (BLC) Urine Leads Concentrations (ULC)

Blood and urine lead concentrations were determined by an experienced technician in the laboratory mentioned above, using graphite furnace atomic absorption spectrometry (Perkin Elmer, Model 3030 with HGA 400 Programmer).

For measuring lead whole blood was used and after clotting with nitric acid, by adding Triton X – 100, the liquid phases was separated from the clot. Ammonium monovanadat 2% in NaOH 1% was then added to neutralize acidity and to prevent graphite tube damage. The solution was centrifuged at high speed for 5 min, and 25 micro liters from the upper layer was injected into the graphite. Thermal program was selected for drying temp; 120 °C, 400 °C for evaporation of organic solvents, 800 °C for inorganic solvents and ash removal, and 1900 °C for atomization. Absorbance was measured at 283.3 nm wavelength. Detection limit was 0.8 µg/l, precision 3.44%, and accuracy 99.4 %. These parameters were established by repeated analyses of biological reference material.

Statistical Package for Social Sciences (SPSS 18, IBM Corporation, and New York, USA) was used for analyzing the laboratory data. Results were expressed as mean ± standard deviation. Association between biochemical and toxicological parameters was evaluated by

Pearson correlation test. Multivariate analysis was performed using linear models and a 2-sided *P* value<0.05 was considered statistically significant.Workers whose blood lead levels were at toxic range were subsequently treated according to standard guidelines [11].

RESULTS

Out of 138 male workers enrolled in the study, 112 completed the investigations. The workers aged 28.78±5.17 yr, weighed 67.10±5.35 kg, and had worked 8.67±1.41 h daily for 3.89±2.40 years. BLC ranged from 109 to 894 µg/L (Mean 398.95±177.41 µg/L) and ULC ranged from 15 to 221 µg/L (83.67±50 µg/l). Correlation between the blood and urine concentrations is illustrated in Figure 1.

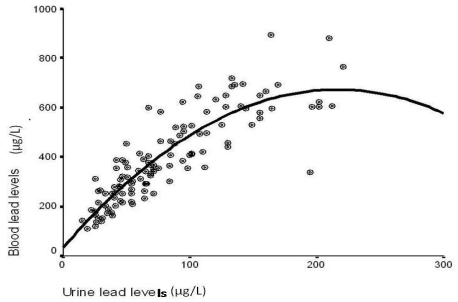

Figure1. Correlation of blood and Urine lead concentrations.

Bivariate correlation showed that there was a significant association (*P*=0.044; r=0.166) between BLC and duration of work among 112 workers. Linear regression analysis revealed that BLC (beta coefficient=0.843; *P*<0.001; r2=0.711) was significantly correlated with ULC. The regression equation was BLC= (3.005×ULC) +147.53. As can be observed in Figure 1, urine lead concentrations increase as blood lead concentrations rise but at higher levels of blood lead concentration, urine concentrations are reduced.

Nonspecific clinical symptoms and signs related to hepatic dysfunction were observed as described in Table 1. The all-hepatic function tests were within the normal ranges.

Correlations between the clinical findings of hepatotoxicity and BLC are described in Table 1. We found no significant statistical relationship between clinical findings of liver damage and BLC (Table 1) or ULC. Likewise, there were no statistically significant relationships between biochemical tests (AST, ALT, Bilirubin Total and Direct, or LDH concentrations) and BLC as described in Table 2. There was a weak correlation between serum level of alkaline phosphatase and BLC (R: 0.217, *P*: 0.087) as shown in Table 2 and Figure 2.

Table 1. Association between clinical findings of hepatotoxicity and BLC. No significant correlation was found.

Variable	Blood lead concentration (µg/L)			
	Positive		Negative	
Symptom/sign	Mean± Std. Deviation	N	Mean± Std. Deviation	N
Palmar erythema	364.57±148.81	7	402.09±180.75	105
Abdominal tenderness	331.00±115.62	6	408.24±179.53	106
Icterus detected in sclera	412.94±191.85	17	397.43±177.12	95
Temporal muscle atrophy	392.66±208.13	3	399.96±178.83	109
Skin hyperpigmentation	393.89±184.95	9	401.00±179.21	103
Dark-colored urine	370.53±138.27	17	401.97±183.61	95
Acholic stool	458.75±68.92	4	397.60±181.14	108
Vomiting	542.50±241.81	4	394.53±175.14	108
Nausea	448.22±212.26	8	395.57±175.96	104
Metal taste in mouth	430.56±160.11	8	386.87±182.92	104
Abdominal pain	339.60±130.98	9	405.61±181.99	103
Fatigue	373.02±152.80	45	413.44±191.40	67
Anorexia	391.27±147.01	28	403.80±189.87	83
Hepatomegaly	-	0	-	112
Splenomegaly	-	0	-	112

Table 2. Correlation between AST, ALT, LDH, Bilirubin Total and Direct, ALP concentrations and BLC.

Variables	Mean Values	Correlation with blood lead level R (P-value)
AST(IU/L)	32.63	-0.093 (0.467)
ALT(IU/L)	32.44	-0.129(0.315)
LDH(IU/L)	387.67	0.092(0.472)
BIL.T(mg/dl)	.824	-0.193(0.130)
BIL.D(mg/dl)	.233	-0.175(0.170)
ALP(IU/L)	118.49	0.217(0.087)

AST: Aspartate Aminotransferase; ALT: Alanine aminotransferase; LDH*: Lactate dehydrogenase*; Bil
T: *Bilirubin total*; Bil D: *Bilirubin direct;* ALP: *Alkaline phosphatase.*

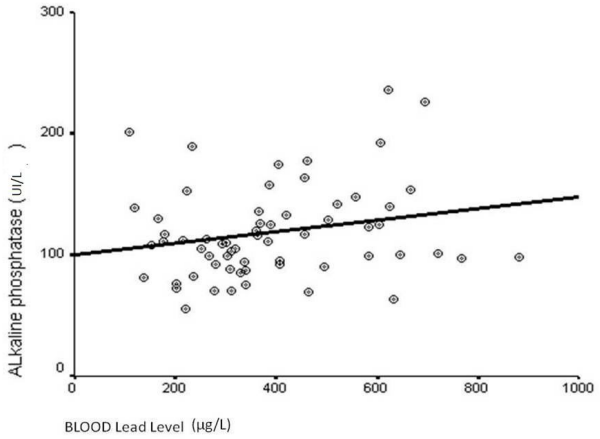

Figure 2. Correlation between blood lead concentration and serum concentration of alkaline phosphatase**.**

DISCUSSION

Lead is found widely in car batteries, paints and building materials, so is ubiquitous all over the world. Liver, as the largest gland of human body, serves multiple main vital functions such as biotransformation of drugs and toxins, metabolism and excretion of bilirubin and cholesterol, production of albumin, coagulation factors and other major plasma proteins. Accumulation of significant amounts of lead results in oxidative stress in liver tissue [12, 13]. Liver has been reported as the largest repository of lead among soft tissues followed by kidney [14]. Several animal studies have been conducted on lead induced hepatotoxicity [3-6] and hepatocyte proliferation, fatty changes, hydropic degeneration, necrosis of the hepatocytes, mild fibrosis, biliary hyperplasia have been described in previous reports [12, 14].

As an environmental exposure to lead is increasing, progressive health risks are being reported. The respiratory and gastrointestinal systems are considered as main entry points of lead exposure in humans [4]. Two main factors are responsible for toxicological effects of lead: concentration and duration of exposure [7]. Car battery workers are particularly at risk for lead exposure. Many processes in battery plants of Iran are performed manually and the release of lead vapors, particles and debris induce considerable environmental pollution, which can result in lead poisoning.

In Netherland, a 40-yr-old Iranian man with regular consumption of opium was referred with severe abdominal pain and anemia due to lead poisoning (12). Lead is a common opium contaminant. His liver enzymes were raised and liver biopsy showed hepatitis, cholangitis and hemosiderosis. A case series of four opium addict patients with lead intoxication demonstrated that headache, vomiting and abdominal pain were common symptoms and liver biopsies showed high lead content and

nonspecific hepatitis. Liver enzymes returned to normal after discontinuation of opium consumption [12]. These cases are considered as acute and sub-acute lead poisonings that are different from the chronic occupational Pb intoxication as observed in our patients.

In the present study, we could not detect any relationship between clinical and laboratory findings of liver damage and BLC or ULC. There was a weak correlation between serum levels of ALP and BLC (R: 0.217, *P*: 0.087).

An investigation on 100 industrial and 100 non-industrial workers in United Arab Emirates showed that the ALP ($P = 0.012$), and LDH ($P < 0.029$) were higher in the exposed workers than in the non-exposed group, although there was no significant difference in transaminases and bilirubin between the two groups. The duration of exposure was longer (Mean: 8.3 ± 5.9 yr) and mean blood lead level in exposed group (775±428 µg/l) was higher as compared with our study [15].

An investigation in India compared 30 lead exposed automobile workers (with routine activities like battery recharging, replacing, welding, spray painting, radiator repairing, brazing etc) with 30 nonexposed healthy man; bilirubin level of serum was significantly increased in the automobile workers as compared to controls (45.83%, $P<0.001$) as well as AST (23.88%, $P <0.001$) and ALT (24.03%, $P < 0.001$). Mean blood lead concentration was 47.37± 23.22 µg/dl, which is slightly higher than our results. The authors suggest that increased levels of serum bilirubin may be due to hemolysis of erythrocytes because of morphological changes that occur in high lead concentrations. Long-term exposure to lead (more than 6 hours per day over 2-20 years) in automobile workers was considered as the reason of increased levels of transaminases. ALP activity was significantly increased in exposed group in comparison with the controls (17.99%, $P < 0.001$) [7].

In India in survey on 30 battery manufacture workers (which involved the use of metallic lead for making grids, bearings), slightly higher serum bilirubin levels were observed in comparison with non-exposed control group. Transaminase levels were slightly higher in exposed group, although these amounts were in acceptable range for the used method. Mean blood lead level was 53.63 ± 16.98 µg/dl. These observations were in agreement with our results, although BLC in our subjects was lower. The authors did not recommend liver function tests as a definitive evidence of lead exposure at the observed BLC range (highest at 78 µg/dl); however, they recommended urinary δ amino levulinic acid level as a more suitable and convenient marker to screen and confirm occupational lead exposure [1].

Dioka, C., et al. conducted a study on 25 occupationally exposed artisans and 25 graduate students of a college of health sciences in Nigeria. Liver function tests including AST, ALT and ALP activity were evaluated for determining toxicological effects of occupational exposure to petroleum products (especially petrol containing tetraethyl lead). Total transaminase activity was elevated in the exposed group as compared with the controls, but ALP activity was significantly decreased and the authors claimed that this was due to decreased zinc levels in exposed artisans. Mean blood lead level was 59.6 ± 15.9 µg/dl in the exposed group [10].

In our study, although AST, ALT, Bilirubin and ALP were at normal range, weak correlation of ALP with BLC might be considered as a result of hepatobilliary disorder and in a lesser degree due hepatocellular damage; although this enzyme could have originated from other sites such as bones and intestine. Increased ALP activity generally originates from hepatobiliary system. Toxic effects of lead on liver could result in disturbances of the transport functions of the hepatocytes or of the biliary tree and can cause elevation of serum ALP activity [7].

CONCLUSION

This study did not reveal objective signs or laboratory abnormalities of liver function in workers who had toxic levels of BLC. We found only a weak correlation between serum level of ALP and blood lead concentration in the exposed workers. Further investigations with more specific laboratory tests such as LDH5 and gamma glutamyl transferase (GGT) as well as novel biomarkers of metal induced hepatotoxicity might be helpful in evaluating lead hepatotoxicity.

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REFERENCES

1. Bhagwat VR, Patil AJ, Patil JA, Sontakke AV. Occupational lead exposure and liver functions in battery manufacture workers around Kolhapur (Maharashtra). Al Ameen J Med Sci 2008;1(1):2-9.
2. Kianoush S, Balali-Mood M, Mousavi SR, Shakeri MT, Dadpour B, Moradi V, Sadeghi M. Clinical, toxicological, biochemical, and hematologic parameters in lead exposed workers of a car battery industry. IJMS 2013 Mar;38(1):30.
3. Kumar MR, Reddy KS, Reddy AG, Reddy RA, Anjaneyulu Y, Reddy DG. Lead-induced hepatotoxicity and evaluation of certain anti-stress adaptogens in poultry. Toxicol Int 2011 Jan;18(1):62.
4. Sharma V, Pandey D. Protective role of Tinospora cordifolia against lead-induced hepatotoxicity. Toxicol Int 2010 Jan;17(1):12.
5. Haleagrahara N, Jackie T, Chakravarthi S, Rao M, Kulur A. Protective effect of Etlingera elatior (torch ginger) extract on lead acetate-induced hepatotoxicity in rats. J Toxicol Sci 2010;35(5):663-71.
6. Abdel-Moneim AE, Dkhil MA, Al-Quraishy S. The redox status in rats treated with flaxseed oil and lead-induced hepatotoxicity. Biol Trace Elem Res 2011 Oct 1;143(1):457-67.
7. Dongre NN, Suryakar AN, Patil AJ, Rathi DB. Occupational lead exposure in automobile workers in North Karnataka (India): effect on liver and kidney functions. Al Ameen J Med Sci 2010;3(4):284-92.
8. Mudipalli A. Lead hepatotoxicity & potential health effects. Indian J Med Res 2007 Dec 1;126(6):518.
9. Aziz II, Al Agha SZ. Hematological and Biochemical Studies for Gasoline Toxicity Among Gasoline Workers In Gaza Strip. Al-Agsa Univ. J 2006;10:41-55.
10. Dioka CE, Orisakwe OE, Adeniyi FA, Meludu SC. Liver and renal function tests in artisans occupationally exposed to lead in mechanic village in Nnewi, Nigeria. Int J Environ Res Publ Health 2004 Feb 29;1(1):21-5.
11. Flomenbaum NE. Goldfrank's toxicologic emergencies-/[ed. by] Neal E. Flomenbaum...[et al.]. New York, NY,[etc.]: McGraw-Hill; 2006.
12. Verheij J, Voortman J, van Nieuwkerk CM, Jarbandhan SV, Mulder CJ, Bloemena E. Hepatic morphopathologic findings of lead poisoning in a drug addict: a case report. J Gastrointestin Liver Dis 2009 Jun 1;18(2):225-7.
13. Daniel EE. Ameliorative Effect of Vitamin C on Serum Liver Enzymes in Lead-Induced Toxicity in Wistar Rats. J Sci 2013 Feb 21;3(1):188-912.
14. Mehana EE, Meki AR, Fazili KM. Ameliorated effects of green tea extract on lead induced liver toxicity in rats. Exp Toxicol Pathol 2012 May 31;64(4):291-5.
15. Bener A, Almehdi AM, Alwash R, Al-Neamy FR. A pilot survey of blood lead levels in various types of workers in the United Arab Emirates. Environ Int 2001 Oct 31;27(4):311-4.