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OCCUPATIONAL LEAD EXPOSURE IN BATTERY MANUFACTURING WORKERS, SILVER JEWELRY WORKERS, AND SPRAY PAINTERS IN WESTERN MAHARASHTRA (INDIA): EFFECT ON LIVER AND KIDNEY FUNCTION

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ABSTRACT

We studied liver and kidney function tests of occupational lead exposed Battery Manufacturing Workers (BMW) (n=30), Silver Jewelry Workers (SJW) (n=30), and Spray Painters (SP) (n=35) and normal healthy subjects (n=35), all 20 to 40 years of age, in Western Maharashtra (India). Venous blood and random urine samples were collected from all groups. The blood lead (Pb-B) and urinary lead (Pb-U) levels were significantly increased in all experimental groups, except urinary lead excretion in SJW as compared with the controls. Liver functions tests parameters (serum transaminase enzymes SGOT, AST, SGPT, ALT) activities were significantly increased only in SP; no alteration was noticed in BMW and SJW as compared with the control group. Serum total protein levels were significantly decreased in all three experimental groups as compared with control subjects. Serum albumin concentrations were significantly decreased in SJW, SP, and increased in BMW. The serum globulin levels, however,

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were significantly decreased, and the albumin/globulin (A/G) ratio was increased in BMW and SJW as compared with the control. The bilirubin level was significantly increased only in BMW. Blood urea was significantly increased only in BMW, and blood urea and serum uric acid were decreased in SJW. The serum creatinine level was not significantly altered in any experimental groups. Increased Pb-B values in all experimental groups indicate the greater rate of lead absorption and impairment of liver and kidney functions in all three types of occupational lead-exposed workers of Western Maharashtra (India).

KEYWORDS

blood lead , urinary lead, BMW, SJW, SP, liver function tests, kidney function tests

INTRODUCTION

Lead poisoning is not only an environmental disease but also a disease of lifestyle. Lead is one of the best studied toxic substances, hence we know more about the adverse health effects of lead than virtually any other chemical. Lead is a ubiquitous and versatile metal that has been used by mankind for over 6000 years and is today one of the most widely distributed toxins in the environment. Lead in the environment can derive from either natural or anthropogenic sources. Lead is a soft, silvery grey metal, melting at 327.5°C, highly resistant to corrosion, pliable, having high density, low elasticity, high thermal expansion, low melting point, easy workability, easily recycled, excellent antifriction metal, and inexpensive /1-2/. Due to these properties, lead is used for various purposes. Lead and its compounds can enter the environment at any point during mining, smelting, processing, use, recycling, or disposal /1/. Lead is mainly used in acid batteries, colored pigments, jewelry industries for making silver rings and soldering, petrol additives (tetra ethyl and tetra methyl), ship construction. The metal is also used in solder applied to water distribution pipes and to the seams of cans used to store food /1-2/.

The routes of exposure for inorganic lead are inhalation and ingestion. Lead fumes and soluble respirable dust are almost completely absorbed by inhalation. Adults absorb approximately 15% of an ingested dose through the gastrointestinal (GI) tract in contrast to 50% GI absorption in children. Gastrointestinal absorption is generally inversely proportional to particle size and directly proportional to the solubility of the lead compounds /1-3/. Dietary factors, nutritional status, and the chemical form of the metal and patterns of food intake affect absorption. Once absorbed, lead is found in all tissues, but eventually > 90% of the body burden accumulates (or is redistributed) into bone, where it remains with a half life of 27 to 30 years. Lead is excreted primarily through the urine (> 90%), lesser amounts are eliminated via the feces, sweat, hair, and nails.

Lead interferes with the function of enzymes and essential cations (particularly calcium) in cells throughout the body /1-3/, and lead poisoning is usually associated with multi-systemic signs and symptoms /4-5/. 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 /1-2/. 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 /1-2 /.

A wide variation exists in individual susceptibility to lead poisoning, with a corresponding range in the spectrum of clinical findings symptoms sometimes appearing in adults with blood lead concentrations as low as 25μg/dL; more commonly, however overt symptoms emerge in patients whose peak blood lead concentration has exceeded 40-60 μg/dL. Subacute or chronic intoxication is more common than acute poisoning. Early symptoms are often subtle, nonspecific, and/or subclinical, involving the nervous system (restlessness, fatigue, irritability, sleep disturbance, headache, difficulty in concentrating, decreased libido), GI system (abdominal cramps, anorexia, nausea, constipation, diarrhea), or musculoskeletal system (arthralgia, myalgia). Other less common conditions include tremor, toxic hepatitis, or acute gouty arthritis (saturnine gout). In general, the

number and severity of symptoms worsen with increasing blood lead levels. A high blood lead level of intoxication may result in delirium, coma, and seizures associated with lead encephalopathy, a life threatening condition /1-3/.

Occupational exposure to lead is entirely unregulated in many developing countries, and little monitoring is conducted in developed countries /6/. Battery recycling and manufacturing is the informal work setting that involves the use of metallic lead for making grids, bearings, and solder. Manufacturing processes are usually manual and involves the release of lead particles and lead oxide that can cause severe poisoning and environmental pollution. Battery recycling is an important source of exposure to inorganic lead vapors, particles, and debris /7/. In silver jewelry industries, lead is used mainly for making and designing the silver rings, and such work is predominantly carried out at home or in non-regulated shops. The lead fumes and dust generated in silver refining units and silver rings making workshops pose an exceptional health hazard to children and adults living near these operations /5, 8/.

Lead is also used in various paints because of its anticorrosive properties. Earlier in India, 10% of total lead metal utilized is used in the manufacture of paints, and wherever such paints are used there will be potential for human lead exposure. Indian paint samples tested had lead concentration exceeding 1% by weight. In India, spray painters and children are at high risk of lead exposure because there is likely to be a reservoir of painted objects in residential setting /9-10/.

Therefore, our aim of this study is to assess the effects of lead exposure and it's toxicity on liver and kidney functions of occupational lead-exposed battery manufacturing and in silver jewelry and spray painting workers in Western Maharashtra (India).

EXPERIMENTAL

The study group included 30 occupational lead-exposed healthy male subjects from battery manufacturing industries, 30 silver jewelry workers, and 30 spray painters of Kolhapur City in Western Maharashtra. All three groups were aged in the range of 20 to 40 years. Thirty-five age-matched non-occupationally lead-exposed normal

healthy control subjects were taken from rural areas. Before the biological specimen collection, the demographic, occupational, and clinical data were collected from the study and control subjects by questionnaire and interview. Male subjects of average socioeconomic status, normal dietary intake and food habits, nonsmokers, and non-alcoholic, who were occupationally exposed to lead for more than 6 hrs per day over 2 to 20 years, were selected for this study. Subjects who were on drugs for minor illnesses and past history of major illness 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 /11/.

Blood was collected by venipuncture into evacuated tubes containing heparin solution as anticoagulant. At the time of blood collection, random urine samples were collected to avoid errors that can occur from the inadequate collection of 24-h urine samples from each subject.

Estimations of lead in blood and urine were carried out by graphite furnace atomic absorption spectrophotometer (AAS) using a Perkin Elemer model 303 fitted with a boiling three slot burner. The AAS was connected to Hitachi 165 recorder and values were shown in µg/dL /12/. The liver and kidney functions tests were measured by using a fully automated biochemistry analyzer (Eurolyser) on the same day of sample collection. The serum transaminases SGOT (AST) and SGPT (ALT) were measured by the UV-kinetic method /13, using reagents from M/S Accurex Biomedical Ltd. The conversion of NADH to NAD in both transaminase (SGOT, SGPT) reactions was measured at 340 nm, as the rate of decrease in absorbance.

Serum total proteins were measured by the Biuret method /14/ 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 /15/ using reagents from M/S Beacon Ltd. Serum albumin binds with 3,3′,5,5′-tetrabromocresol sulfonapthalein (BCG) in acidic medium at pH 4.2, and the blue-green colored complex formed is measured at 600 nm. Serum globulin and

the A/G ratio were calculated by using serum total proteins and albumin values.

Serum total bilirubin was estimated by the Jendrassik method /16/ 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.

Blood urea was measured by GLDH method /17/ using an M/S Agappe 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 /14, 18/ using an M/S Accurex biomedical kit. Serum creatinine in alkaline medium reacts with picrate to produce orange color that absorbs light at 492 nm. The rate of increase in absorbance is directly proportional to the concentration of creatinine in specimen.

Serum uric acid was measured by the Uricase/PAP method /19/ using a Crest Bio system kit. Uricase converts uric acid to allantoin and hydrogen peroxide. The H_2O_2 formed further reacts with a phenolic compound and 4-amino antipyrine by the catalytic action of peroxidase to form a red colored quinoneimine dye complex. The intensity of the color formed is directly proportional to the amount of uric acid present in the sample.

Statistical analyses between the control and BMW, SJW, and SP groups were carried out using the unpaired Students *t* test.

RESULTS

Table 1 summarizes the lead concentration in blood (Pb-B) and urine (Pb-U), and all parameters related to liver function of all groups. The blood lead and urinary lead levels were significantly increased in all experimental groups, except urinary lead excretion in SJW as compared with their respective control groups. The SGOT and SGPT

TABLE 1

Pb-B, Pb-U and parameters related to liver functions in experimental and control groups

Parameter	Group			
	Control (N =35)	BMW (N =30)	SJW (N =30)	SP (N =30)
Pb-B (µg/dL)	12.52 ± 4.08 (2.8-22.0)	53.63±16.98§ (25.8-78.5)	48.56 ±7.39§ (30.2-64.7)	22.32± 8.87§ (7.5-45.7)
Pb-U (µg/dL)	6.97±3.59 (1.0-13.2)	20.04±15.21§ (5.2-62.8)	9.39 ± 6.52^{NS} (2.0-30.7)	11.22 ± 7.15‡ (1.5-30.2)
SGOT (U/L)	30.02±7.77 (11.14-38.58)	32.61±8.25^{NS} (15.32-44.40)	31.20±15.13^{NS} (8.88-73.18)	41.52 ±17.3‡ (13.13-83.03)
SGPT (U/L)	15.94±8.93 (4.69-35.18)	17.27±7.19^{NS} (7.19-26.41)	22.40±15.20^{NS} (3.53-52.48)	23.23±11.62‡ (8.13-44.91)
Serum total protein (g/dL)	8.01 ± 0.49 (6.88-8.89)	7.70 ± 0.56* (6.75-8.43)	7.46 ± 0.80‡ (6.16-9.24)	7.63 ± 0.53‡ (6.87-8.83)
Serum albumin (g/dL)	4.40 ± 0.18 (4.11- 4.73)	4.60 ± 0.11§ (4.35-4.93)	4.25±0.17‡ (3.90-4.62)	4.27±0.15‡ (3.83-4.59)
Serum globulin (g/dL)	3.61±0.44 (2.7-4.58)	3.10 ± 0.54§ (2.03-4.45)	3.21 ± 0.87** (1.67-5.11)	3.36±0.50^{NS} (2.75-4.61)
A/G ratio	1.23±0.16 (0.9-1.75)	1.53 ±0.29§ (1.03-2.69)	1.43±0.45** (0.8-2.68)	1.29±0.18^{NS} (0.91-1.60)
Serum total bilirubin (mg/dL)	0.73±0.32 (0.40-1.39)	0.97±0.40** (0.53-2.03)	0.91±0.48^{NS} (0.23-2.12)	0.76±0.29^{NS} (0.34-1.51)

BMW, battery manufacturing workers; SJW, silver jewelry workers; SP, spray painters. $^{\S}p < 0.001$, $^{\ddagger}p < 0.01$, $^{**}p < 0.02$, $^{*}p < 0.05$, NS, Not significant.

levels were significantly increased only in spray painters, and no significant differences were observed in BMW and SJW as compared with their respective control groups. The serum total protein levels were significantly decreased in all three experimental groups as compared with the respective control subjects. The serum albumin

TABLE 2

Parameters related to kidney functions of experimental and control group

Parameter	Group			
	Control (N =35)	BMW (N =30)	SJW (N =30)	SP (N =30)
Blood urea (mg/dL)	25.12 ± 5.73 (14.20-36.93)	30.43±11.04* (17.61-58.52)	19.97±5.84‡ (8.19-35.0)	26.67±8.34^{NS} (14.21-44.74)
Serum Uric Acid (mg/dL)	5.57 ± 0.97 (4.05-7.69)	5.92±0.95^{NS} (4.08-7.54)	4.07±1.01§ (2.06-5.87)	4.90±1.10** (2.77-8.05)
Serum creatinine (mg/dL)	0.81±0.11 (0.53-1.07)	0.83±0.15^{NS} (0.57-1.11)	0.83±0.20^{NS} (0.41-1.41)	0.88±0.22^{NS} (0.45-1.283)

BMW, battery manufacturing workers; SJW, silver jewelry workers; SP, spray painters. §p < 0.001, ‡ p < 0.01, ** p < 0.02, * p< 0.05, NS, Not significant.

concentrations were significantly decreased in SJW, SP and increased in BMW. Serum globulin levels were significantly decreased, however, and the A/G ratio increased in BMW and SJW as compared with the controls. The serum total bilirubin level increased only in BMW as compared with control subjects.

Table 2 depicts the parameters related to the kidney functions of experimental and control groups. The blood urea level was significantly increased only in BMW. Decreased blood urea and uric acid was observed in SJW as compared with controls. The serum creatinine level was not significantly altered in all three experimental groups as compared with their respective control groups.

The percentage change of Pb-B, Pb-U, and parameters related to liver and kidney functions in BMW, SJW, SP, and control groups are shown in Table 3.

TABLE 3

Percentage change of Pb-B, Pb-U and parameters related to liver and kidney functions of experimental groups compared to control group

Parameter	Group (Percent change)		
	BMW (N =30)	SJW (N =30)	SP (N =30)
Pb-B	+328.35	+287.85	+78.27
Pb-U	+187.51	+34.72	+60.97
SGOT	+8.62	+4.0	+38.30
SGPT	+8.34	+40.52	+45.73
Bilirubin	+32.87	+24.65	+4.10
Total protein	-3.87	-6.86	-4.74
Albumin	+4.54	-3.40	-2.95
Globulin	-14.12	-11.08	-6.92
A/G ratio	+24.39	+16.26	+4.87
Urea	+21.13	-20.50	+6.17
Uric Acid	+6.28	-26.92	-12.77
Creatinine	+2.46	+2.46	+8.64

DISCUSSION

The finding that blood lead (Pb-B) and urinary lead (Pb-U) levels of three study groups were significantly increased as compared with the control group while urinary lead excretion in SJW was not significantly different from controls indicates a greater absorption of

lead in all three study groups than in the control group /4-5/. The absorption of lead ordinarily results in rapid urinary lead excretion. If excessive lead exposure continues, lead accumulates in bone. If bone storage capacity is exceeded, then lead shifts to the soft tissues. The blood lead level depends on the equilibrium between absorption, storage, and excretion. The Pb-B level is generally reflects acute (current) exposure but is also influenced by previous storage /1-2/.

Urine lead excretion has also been employed as an index of exposure because blood lead values change more rapidly than urine lead excretion does. Excretion values depend on 24 hour urine specimens, however, with the usual difficulty in complete collection. The Pb-U estimation is useful test of lead absorption because Pb-U reflects Pb-B fairly well. Yet, Pb-U is not as suitable as Pb-B because numerous factors other than the degree of lead absorption alone, such as renal function, fluid intake, and specific gravity of the urine influence the urinary excretion of lead /20/. After the administration of a chelating agent like Ca Na_2-EDTA, the Pb-U level is considered an excellent measure of the potentially toxic fraction of the total body burden of lead /1-2/.

The Pb-B values found in the present study show that the absorption of lead was more pronounced in BMW and SJW than in SP. The probable reason for this might be less use of lead in paints or could be due to the ban on addition of lead to paints. Lead is used in various paints because of its anticorrosive properties. Previously in India, 10% of the total lead metal utilized was used in the manufacture of paints. Nowadays, lead-free or low-lead content paints are available in the market. Spray painters and children are at more risk of lead exposure because there is likely to be a reservoir of painted objects in residential setting in India /10/. Although the urinary lead level was significantly increased in BMW and SP groups as compared with the control, the absence of a significant difference in SJW the group could be due to a greater excretion of lead through sweating because silver refining unit workers are exposed to more humid and high environmental temperature /5/. The entire work place is very hot due to inadequate ventilation and no chimney is fixed at the silver refining unit. The SGOT (AST) and SGPT (ALT) levels were significantly increased only in spray painters, but no significant difference was observed in BMW and SJW as compared with the

control. As reported in several studies, transaminase enzymes levels are not increased in cases of low to moderate lead absorption /21-22/.

Although the Pb-B level was high in BMW and SJW, the transaminase enzymes levels were not altered. Conversely, the transaminase levels were increased even at low Pb-B levels in spray painters; hence, the increased enzymatic activity could not be due to lead exposure alone. Such an increase might result from the hepato-toxic effect of the solvents (xylene and toluene) used for spray painting. Thus, a detailed study is required to determine the cause of increased transaminase activity in this experimental group.

The mean values of serum total protein, albumin, globulin, and the A/G ratio of all study groups indicate a decreased rate of protein synthesis, mainly albumin and globulin at high Pb-B exposure (BMW and SJW groups). The increased A/G ratio in these two experimental groups could have been due more to a fall of globulin synthesis rather than to an increase in albumin at high Pb-B levels. The absence of a change in the A/G ratio in SP group could reflect the low Pb-B level in this group. In lead-exposed experimental animals, decreased protein synthesis at high Pb-B levels has been reported in several studies /23/. Therefore, the estimation of total protein is also a valuable tool for detecting impairment of liver function in workers having high lead exposure.

An increased serum bilirubin level and the excretion of stercobilinogen and urobilinogen in lead poisoning cases was reported in several studies, concluding that the lead-induced anemia was the hemolytic type /24-26/. Hemolytic jaundice and raised serum bilirubin levels have been reported in several cases of lead poisoning /27-28/. High concentrations of lead have been shown to produce morphological changes and destruction of red blood cells when administered in vitro or vivo /29/. This study demonstrates that the rate of hemolysis is greater at high Pb-B levels. Hence, the serum bilirubin level was increased only in BMW (high lead exposure group) as compared with the control group.

From the results of serum urea, uric acid, and creatinine levels, the impairment of kidney functions in the SP group appears to be the lowest in comparison with the BMW and SJW groups. Lead is known to cause 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 /1-2/. Tubular effects are noted after relatively short-term exposure and are generally reversible, whereas sclerotic changes and interstitial fibrosis, resulting in decreased kidney functions and possible renal failure, require chronic exposure to high lead levels. Increased risk from nephropathy was noted in workers with a Pb-B level of over 60 µg/dL /1-2/.

Chronic nephropathy, which may progress to kidney failure, is the classic renal manifestation of lead toxicity. The cell lining of the proximal tubules in the kidney is highly sensitive to lead. Lead induces the formation of dense intranuclear inclusion bodies consisting of lead protein complex found at Pb-B concentration of 40-80 µg/dL. Hyperuricemic gout apparently resulting from the increased reabsorption of uric acid by the tubular cells is a third metabolic complication of lead-induced renal impairment /1, 9/. As the clinical manifestations of impairment, characterized by a rise in blood urea nitrogen or serum creatinine, do not ordinarily become evident until 50% to 70% of the nephrons are destroyed /2, 8/, the estimation of blood urea, uric acid, and creatinine may not be useful for screening low lead exposure.

The increased Pb-B values in the experimental groups as compared with the control group indicate that despite modern technical advancements, the rate of lead absorption was definitely greater in all three experimental groups. The increased lead absorption impaired the liver and kidney functions of all three occupationally lead-exposed groups, particularly in the BMW and SJW groups.

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