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ORIGINAL ARTICLE

Occupational Lead Exposure In Automobile Workers In North Karnataka (India): Effect On Liver And Kidney Functions

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Abstract: We studied liver and kidney function tests of occupational lead exposed Automobile Workers (N = 30), and normal healthy control subjects (N = 30), all 20 to 45 years of age, from Bijapur, North Karnataka (India). Venous blood and random urine samples were collected from both groups. The blood lead [PbB] (364%) and urinary lead [PbU] (176%) levels were significantly increased in automobile workers as compared with the controls. Liver function test parameters, i.e. Serum Aspartate Transaminase [AST] (23.88%), Alanine Transaminase [ALT] (24.03%), Alkaline Phosphatase [ALP] (17.99%), Total Bilirubin (45.83%), and Gamma glutamyl Transferase [GGT] (44.75%) were significantly increased in automobile workers as compared with the control group. Serum total protein, albumin, globulin, and A/ G ratio were not significantly altered in study group as compared with control subjects. In the kidney function tests levels of blood urea (26%), serum uric acid (13.11%) and serum creatinine (12.5%) were significantly increased in automobile workers as compared to control group. Increased PbB values in study group indicate the greater rate of lead absorption and impairment of liver and kidney functions in occupational lead-exposed automobile workers from Bijapur, North Karnataka (India).

Keywords: Blood lead, Urinary lead, Automobile Workers, Liver and Kidney function tests.

Introduction

Lead has been used by humans for at least 7000 years [1]. It is highly resistant to corrosion, pliable, having high density, low elasticity, high thermal expansion, low melting point, easy workability, easily recycled, and excellent antifriction metal and inexpensive, due to its excellent properties used in acid battery manufacture, printing press, silver jewellery making, soldering cans, traditional practices such as folk remedies, cable sheathing, in colour pigments, petrol additives, soldering water distribution pipes, ceramic glazes, paper industries. Lead and its compounds can enter the environment at any point during mining, smelting, processing, use, recycling or disposal [2-5]. Health risks are increasingly associated with environmental exposures to lead emissions from the wide spread use of lead in industrial set up [6]. A human exposure to lead is mainly through the air, food, dust, soil and water. The inhalation and ingestion are the primary roots of absorption of lead compounds. Approximately 40% of lead oxide fumes are absorbed through respiratory tract and 5-10% absorbed from the gastro-Intestinal tract. In blood 98% lead is mainly bound to erythrocytes and 2% present in plasma, which can be distributed to brain, kidney, liver, skin and skeletal muscles where it is readily

exchangeable [7]. The ingested and absorbed lead is mainly stored in soft tissues and bones. Autopsy studies of lead exposed humans indicate that liver is the largest repository (33%) of lead among the soft tissues followed by kidney, cortex and medulla (8). Lead is excreted primarily through the urine (> 90%), lesser amounts are eliminated via the feces, sweat, hair, and nails. Lead has been shown to cause adverse effects on several organs and organ systems, including the hematopoietic, nervous, renal, cardiovascular, reproductive, and immune system and is mutagenic in mice [3-5]. The biological effects of lead depend upon the level and duration of exposure. Sub acute or chronic intoxication is more common than acute poisoning. Clinical symptoms of lead toxicity are restlessness, fatigue, irritability, sleep disturbance, headache and difficulty in concentrating, decreased libido, abdominal cramps, anorexia, nausea, constipation, and diarrhea. Other less common conditions include tremor, toxic hepatitis, or acute gouty arthritis. 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 [9-10]. Lead 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. Lead also affects normal liver functions, impairs the detoxification of xenobiotics (environmental toxins and drugs) [11]. Therefore, our aim of this study is to assess the effects of lead exposure and its toxicity on liver and kidney functions of occupational lead-exposed automobile workers from Bijapur, North Karnataka (India).

Materials and Methods

Study group consisted 30 occupationally lead exposed healthy males from automobile workshops and 30 non occupationally lead exposed healthy male subjects were taken from the educational institutes of Bijapur city as controls. Both group subjects were aged in the range of 20-45 years. Before sample collection, the demographic, occupational and clinical data were collected from the control and study group subjects by questionnaire and interview. Male subjects of average socio-economic status, normal dietary intake and food habits, non-smokers, non-alcoholic who were occupationally exposed to lead for more than 6 hrs per day over 2-20 years were selected for the study. Most of the workers consumed mixed type of diet. The entire protocol was approved by the institutional ethical committee. Blood was collected by venipuncture into evacuated tubes and EDTA tubes. At the time of blood collection, random urine samples were collected to avoid errors from inadequate collection of 24 hrs urine sample from each subject into dark brown and amber coloured bottles. Estimations of lead in blood and urine were carried out by graphite farness atomic absorption spectrophotometer (AAS) using a Perkin Elmer model 303 fitted with a boiling 3 slot burner. The AAS was connected to Hitachy 165 recorder and values were shown in microgram per liter [12]. The liver and kidney function tests were measured on semiautoanalyzer on the day of sample collection. SGOT (AST) and SGPT (ALT) were measured by the UV kinetic method.

The conversion of NADH to NAD in both transamination reactions were measured at 340 nm as the rate of decrease in the absorbance [13]. Serum total protein was measured by biuret method. Protein reacts with cupric ion in alkaline PH to produce a purple coloured complex. The intensity of the colour complex was measured at 546 nm and directly proportional to the concentration in the sample [14]. Serum albumin was measured by BCG method. Serum albumin binds with 3-3', 5-5' – tetrabromocresol green (BCG) in acidic medium at pH 4.2, and blue green coloured complex formed is measured at 600 nm. Serum globulin and A/G ratio were calculated by using serum total proteins and albumin values [15]. Serum total bilirubin was estimated by Jendrassik method. Serum bilirubin reacts with the diazotized sulphanilic acid to produce azobilirubin (pink colour). Di-methyl sulphoxide (DMSO) catalyzes the formation of azobilirubin from free bilirubin. The intensity of pink colour is proportional to the bilirubin concentration measured at 546 nm [16]. Blood urea was estimated by GLDH method. Urea is decomposed by urease to form ammonia and CO_2. Ammonia combines with 2-oxo-glutarate in presence of glutamate dehydrogenase and NADH to form L- Glutamate and NAD. The rate of NAD formation measured at 340 nm is directly proportional to the amount of blood urea. Each molecule of urea hydrolyzed liberates two molecules of NAD^+ [17].

Serum creatinine was estimated by Jaffe's method. Serum creatinine in alkaline medium reacts with Picric acid to produce orange colour that absorbs light at 492 nm. The rate of increasing absorption is directly proportional to the amount of creatinine in the sample [18]. Serum uric acid was measured by the uricase/PAP method using . Uricase converts uric acid to allantoin and hydrogen peroxide. The hydrogen peroxide formed further reacts with a phenolic compound and 4 amino antipyrine by the catalytic action of peroxidase to form a red coloured quinonimine dye complex. The intensity of the colour formed is directly proportional to the amount of uric acid present in the sample [19]. Serum ALP (EC 3.1.3.1) was estimated by the kinetic method using paranitrophenyl phosphate (PNP). Alkaline phosphatase cleaves paranitrophenyl phosphate into paranitrophenyl and phosphate. Paranitrophenyl is a yellow colour compound in alkaline medium and absorbs light at 405nm. The rate of increase in absorbance at 405nm is proportional to the ALP activity in the sample [20-21]. Serum GGT (EC 2.3.2.2) was estimated by the kinetic method using reagents from Raichem. GGT catalyzes the transfer of glutamyl group from L-γ glutamyl-3-carboxy-4nitro anilide to glycyl glycine with the formation of L-γ glutamyl glycine and 5-amino-2nitro benzoate. The substrate (L-γ glutamyl-3-carboxy-4nitro anilide) has no colour. The product 5-amino-2nitro benzoate absorbs strongly at 405 nm. The amount of 5-amino-2nitro-benzoate liberated is proportional to GGT activity and is measured kinetically at 405 nm by the increasing intensity of the yellow colour formed [22]. Statistical analysis was done using students't' test.

Results

Table 1: Mean values of liver and kidney function tests of automobile workers and control group.

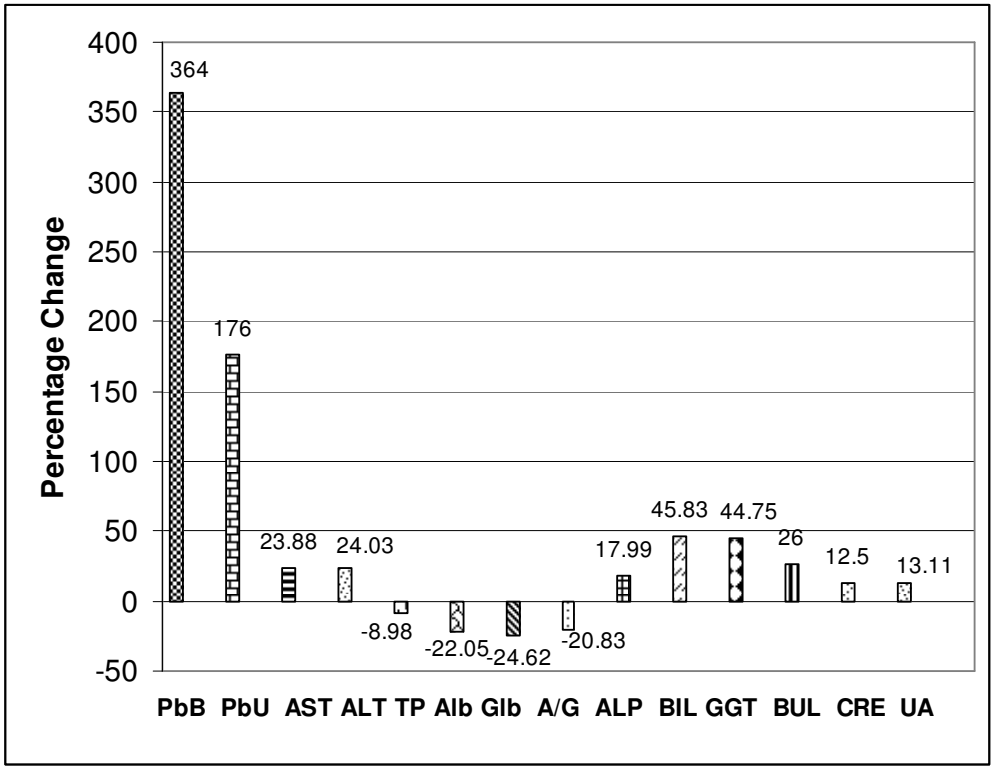
Sr. No.	Biochemical Parameters	Control group (N = 30)	Automobile workers (N = 30)
1.	PbB µg/dl	10.2 ± 5.8 (2.0 - 23.0)	47.37± 23.22*** (5.0 - 85.0)
2.	PbU µg/dl	6.28 ± 3.83 (1.0 - 14.0)	17.37± 12.5*** (1.0- 41.0)
A	Liver functions tests		
1	Aspartate Transaminase [U/L]	27.92 ± 9.37 (22 – 54)	34.59 ± 11.66* (15 – 60)
2	Alanine Transaminase [U/L]	33.08 ± 8.46 (17 – 52)	41.03 ± 18.28* (20-65)
3	Total Protein [gm/dl]	6.57 ± 0.5 (5.9 – 7.30)	5.98 ± 0.6*** (5.9 – 7)
4	Albumin [gm/dl]	3.9 ± 0.35 (2.9 – 3.9)	3.04 ± 0.28*** (2.8 – 4.4)
5	Globulin [gm/dl]	3. 98 ± 0.30 (2.5 – 3.9)	3.0 ± 0.21*** (2.1 – 4.3)
6	Albumin/Globulin Ratio	1.2 ± 0.09 (0.79 – 1.76)	0.95 ± 0.08*** (0.8 – 1.1)
7	Alkaline Phosphatase [U/L]	125.84 ± 18.44 (105 – 150)	148.48 ± 26.83*** (110 – 180)
8	Bilirubin [mg/dl]	0.96 ± 0.25 (0.5 – 1.4)	1.4 ± 0.33*** (0.7 – 2.2)
9	Gamma Glutamyl transferase	26.12 ± 7.38 (18-43)	37.81 ± 11.09*** (22-70)
B	Kidney functions tests		
1	Blood Urea [mg/dl]	24.03 ± 3.69 (17 – 30)	30.29 ± 5.17*** (16 – 52)
2	Serum Creatinine [mg/dl]	1.04 ± 0.13 (0.9 – 1.3)	1.17 ± 0.316* (0.8– 2.2)
3	Serum Uric Acid [mg/dl]	5.26 ± 0.834 (2.9 – 6.6)	5.95 ± 1.04** (3.7– 7.4)

Figures indicate Mean ± SD values and those in parenthesis are range of values of the present study groups. * P < 0.05, ** P < 0.01, *** P < 0.001, • Non significant as compared to controls.

Blood lead and urinary lead levels were significantly increased in the automobile workers as compared to the control subjects .The mean and SD values of lead in blood and urine in automobile workers were significantly increased that is PbB 47.37± 23.22 µg/dl (364%) and PbU 17.37± 12.5 µg/dl (176 %) as compared to controls. Mean values, SD and Range of biochemical parameters of PbB, PbU, liver and kidney function, of automobile workers and the controls are shown in Table -1.

The AST (23.88%), ALT (24.03%), ALP (17.99%), and GGT (44.75 %) levels were significantly increased in automobile workers as compared to the controls. Serum total proteins (-8.98%), serum albumin (-22.05%), globulins (-24.62%), and A/G ratio (-20.83%) levels were significantly decreased in automobile workers as compared to the control subjects. Serum total bilirubin (45.83%), blood urea (26%), serum creatinine (12.5%) and serum uric acid (13.11%) levels were significantly increased in automobiles workers as compared to the controls. (Fig.-1)

Fig 1: Percentage change PbB, PbU, liver and kidney function tests of automobile workers with respect to control group



PbB – Blood lead, **PbU-** Urinary Lead, **AST-** Aspartate transaminase, **ALT** - Alanine transaminase, **TP** - Total proteins, **ALB** - Albumin, **GLB** - Globulin, **ALP** – Alkaline Phosphatase, **BIL** – Bilirubin, **GGT** – Gamma Glutamyl Transferase, **BUL** - Blood Urea Level , **CRE** – Creatinine**, UA** - Uric Acid.

Discussion

In automobile workers blood lead (PbB) (364%, $P < 0.001$), and urinary lead (PbU) (176%, $P < 0.001$) levels were significantly increased as compared to control subjects, indicates absorption of lead is more in this study group. Absorption of lead ordinarily results in rapid urinary excretion. It is seen that PbB levels generally reflect acute/recent/current exposure and it is also influenced by previous storage. Automobile workers are prone to lead exposure due to their routine activities like

battery recharging, replacing, welding, spray painting, radiator repairing, brazing etc (23). In this study mainly we have taken workers involved in the radiator repairing, spray painting and battery recycling and recharging. The work places were unhygienic and the workers were unaware of the ill effects of the lead exposure. The most common symptoms in workers observed were anorexia, muscular pain, abdominal pain and headache. Increased blood lead level in this study affects on several biochemical parameters. We found significantly increased serum total bilirubin level (45.83%, P<0.001) in the automobile workers as compared to control subjects, may be due to more haemolysis of red blood cells. High lead concentration produces morphological changes and destroys the red cells when administered in vitro and in vivo reported in several studies [24-25]. Serum protein level is a gross measure of protein status and reflects major changes in the liver functions. In this study we observed significant decrease in the serum total proteins (-8.98%, p<0.001), albumins (-22.5%, p<0.001), globulins (-24.62%, p<0.001) and A / G ratio (-20.83%, p<0.001) as compared to controls. The effect of lead exposure on the serum protein levels is controversial. Pachathundikandi et.al observed no change serum total protein level in automobile workers but not by others (23). C. E. Dioka et. al (2004) reported no change in the serum albumin levels in occupationally lead exposed artisans (26). In automobile workers serum albumin showed significant change. It indicates that lead exposure in this study affects the synthetic function of the liver. It is also observed that globulin level significantly deceased in this study that has resulted in decreased A/G ratio. Aspartate transaminase (23.88%, P <0.001) and Alanine transaminase (24.03%, P < 0.001) levels were significantly increased in automobile workers as compared to controls, indicates hepatocellular damage. In several studies it is reported that transaminase enzymes levels are not increased in cases of low to moderate lead absorption. However, increased levels of transaminase enzymes in this study may be due to prolonged duration of exposure to lead in these workers. Lead may accumulate in liver and expert its toxic effect via per oxidative damage to hepatic cell membranes causing transaminase to liberate into the serum [25]. Alkaline phosphatase (17.99%, P < 0.001) level was significantly increased in automobile workers as compared to controls. In this study increased activity of ALP may be due to hepatocellular or hepatobillary injury. Similar results have been also reported in several other studies [25]. Serum ALP activity may originate from liver, bone, intestine or placenta. The increased ALP activity generally shows that the source is hepatobiliary. Toxic liver injury results in disturbances in the transport functions of the hepatocytes or of the biliary tree may cause elevation of serum ALP activity. Assay of ALP activity in serum of anicteric individuals is particularly useful in detecting and monitoring suspected metal induced cholestasis [27-28]. Serum GGT is considered a more sensitive indicator than amino transferase in drug, virus, chemical and alcohol induced hepatocellular damage. Because of its lack of specificity it is to be interpreted in conjunction with other tests [27]. Serum GGT (44.75%, P <0.001) level was significantly increased in this study group as compared to controls. GGT is found on the surface of all cells with particularly high concentration in the liver, bile ducts and kidney. It is mainly involved in the transfer of amino acids across cellular membrane. Also it is involved in the glutathione

metabolism by transferring the glutamyl moiety to a variety of acceptor molecules including water and certain L-amino acids and peptides leaving cysteine products to preserve intracellular homeostasis of oxidative stress. However recent experimental studies indicate that ectoplasmic GGT may also be involved in the generation of oxidative stress. This effect of GGT seems to occur when GGT is expressed in the presence of Fe or other transition metals. The increased GGT levels in the lead induced toxicity may be useful as a marker of oxidative stress. A GGT mediated oxidative stress has been reported, capable of inducing oxidation of lipids, protein thiols, alterations of the normal protein phosphorylation patterns and biological effects such as activation of transcription factor [29]. Blood urea (26%, $P < 0.05$), serum uric acid (13.11%, $P<0.01$) and creatinine (12.5%, $p<0.001$) levels were significantly increased in automobile workers as compared to controls, indicates slight nephrotoxicity may be due to lead.

Conclusions

The study shows that automobile workers must be considered as risky personnel as their routine activities affect many systems in our body like liver, kidney and may lead to organ damage. The dietary factors, nutritional status, patterns of food intake, demographic changes and the chemical form of the metal affects the absorption of lead. The outfits of automobile workshop workers serve as a source of lead exposure to their family members also. Drastic increase in the number of automobile vehicles in last two decades increased the exposure of this labor class to lead .The increased PbB values in the workers indicate that despite modern technical advancements the rate of lead absorption is definitely high in automobile workers. The degree of PbB roughly correlates with the duration of exposure. The study indicates lead toxicity still persists in automobile workers. The study helps to create awareness about the toxic effects of lead and may entail establishment of regulations for the precautionary measures to be taken among the lead exposed workers.

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