

Liver and Renal Function Tests in Artisans Occupationally Exposed to Lead in Mechanic Village in Nnewi, Nigeria

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Abstract: Additives in petroleum solvents have been reported to have adverse health implications. An evaluation study on some toxicological effects of occupational exposure to petroleum products (especially petrol which contains tetraethyl lead) amongst twenty five occupationally exposed artisans and twenty five graduate students of College of Health Sciences, Nnamdi Azikiwe University, Nnewi, Nigeria as controls, was carried out using the following biochemical markers: electrolytes, urea, uric acid, inorganic phosphorus, creatinine, zinc and blood lead, as well as the activities of alanine and aspartate aminotransferases, and alkaline phosphatase. The results showed that occupational exposure of human subjects to lead in petrol increases the concentrations of uric acid ($357 \pm 123 \mu\text{mol/L}$) and phosphate ($1.5 \pm 0.5 \text{m mol/L}$) in exposed subjects compared with unexposed subjects (uric acid $228 \pm 105 \mu\text{mol/L}$, phosphate $1.2 \pm 0.41 \text{m mol/L}$; $p < 0.01$ in both cases). Significantly lower activities were observed for alkaline phosphatase ($66 \pm 18.9 \text{iu/L}$). The activities of alanine aminotransferase ($11.4 \pm 4.0 \text{iu/L}$) and aspartate aminotransferase ($15.8 \pm 4.4 \text{iu/L}$) in occupationally exposed artisans were higher compared with unexposed subjects (alkaline phosphatase = $78 \pm 22.4 \text{iu/L}$ alanine aminotransferase = $6.8 \pm 2.7 \text{iu/L}$, aspartate aminotransferase = $9.6 \pm 3.5 \text{iu/L}$; $p < 0.01$ in all cases). Occupational exposure of human subjects to lead significantly increased blood lead ($59.6 \pm 15.9 \mu\text{g/dL}$) and decreased plasma zinc ($71.3 \pm 14.4 \mu\text{g/L}$) in exposed compared with unexposed subjects (blood lead = $35 \pm 7 \mu\text{g/dL}$, zinc = $108.4 \pm 16.9 \mu\text{g/dL}$; $p < 0.01$). The results indicate that occupational exposure to lead in petrol may compromise liver and renal function.

Key words: Nigeria, Artisans, petroleum, lead, liver, kidney, biomarkers

Introduction

Many compounds such as industrial, agricultural and other environmental chemicals, naturally occurring substances and drugs, adversely affect the kidney. Lead is a toxic metal that is not essential for nutrition [1]. Lead is one of the most widespread potential chemical contaminants in the environment and may be transferred

to man through food [2]. The level of lead in the environment increases proportionately with the level of most useful metals in industry, it has no known biologic function in both animals and man [3].

Nnewi, where this study was done, is a fast growing city in Anambra state, South-Eastern Nigeria. It is highly industrialized. A major problem for Nnewi is the lack of or total absence of the means of disposal of hazardous

wastes. High soil lead levels above 600 ppm have been reported in most of the areas around industries [4].

Because excessive amounts of lead may displace essential trace elements and cause nephropathy and liver dysfunction, [5] renal and liver function tests were determined in artisans occupationally exposed to lead to assess the level of toxicity in these subjects.

Materials and Methods

Subject Selection

The subjects consisted of twenty-five males, aged 25-50 years. (39 ± 8.47) made up of eighteen auto mechanics, five battery chargers and two welders. The group has been constantly exposed to petroleum products. Informed consent was obtained from all subjects after being educated on the benefit of the study.

Control Subjects

Twenty-five graduate students of the College of Health Sciences, Nnamdi Azikiwe University, Nnewi Campus, Nigeria served as controls. They were age matched with the study population and were apparently healthy. Their informed consent was also obtained as for the study subjects.

Determination of Uric Acid

Uric acid was determined by the method of Henry, Sorbel and Kim [6]. All reagents and chemicals were obtained from Cromatest Laboratories, Knickerbocker, Barcelona, Spain. Two hundred μL samples, standard and control were each added to 1.5 mL of uric acid buffer and incubated for 5 minutes at 25°C . Chromogenic mixture (1.5 mL) was added to all tubes, mixed by inversion and incubated for 20 minutes at 25°C . Absorbances were taken at 680 nm using an RA-50 spectrophotometer (Ames/Technicon, France).

Determination of Inorganic Phosphorus

Inorganic phosphorus was determined by the method of Drewes [7]. All reagents were obtained in a ready-to-use form from Cromatest (Spain). Fifty μL of samples, standard and control were added to 2.0 mL of working reagent and incubated for one minute at 25°C . One millilitre of developer was added to all tubes and the mixture allowed to stand at room temperature for 10 minutes. Absorbances of samples, standard and control were determined at 680 nm using a RA-50 spectrophotometer (Ames/Technicon, France).

Determination of Electrolytes, Urea and Creatinine

Serum levels of sodium and potassium were determined using the Corning 410 Clinical Flame photometer method. Chloride and bicarbonate were

determined by titrimetric methods of Schales and Schales [8]. Urea and creatinine were determined by spectrophotometer following the methods of Coloumbe and Farreau [9] and Taussky [10] respectively.

Determination of Alkaline Phosphatase Activity

Alkaline phosphatase activity was determined by the method of Bessey, Lowry and Brook [11]. Reagents for the assay were obtained from Cromatest (Spain) in a ready-to-use form. Samples, standard and controls were incubated with p-nitrophenyl phosphate as substrate for 15 minutes at 37°C . The reaction was stopped by the addition of 5.0 mL of 0.5 M NaOH and the absorbance read at 405 nm using a RA-50 spectrophotometer (Ames/Technicon, France).

Determination of Alanine (ALT) and Aspartate Aminotransferase Activities (AST)

The activities of ALT and AST were determined by the method of Reitman and Frankel [12]. All reagents for the assay were obtained from Cromatest (Spain). ALT and AST substrates (500 μL) were preincubated at 37°C for 5 minutes. 100 μL of samples and control were added and the mixture further incubated for 30 minutes (ALT) and 60 minutes (AST) respectively. The reaction was terminated by adding 500 μL of 1 mmol 2,4-dinitrophenylhydrazine and allowed to stand at room temperature for 20 minutes. The colour was developed by addition of 5 mL of 0.4 M NaOH and absorbance read at 505 nm using a RA-50 spectrophotometer (Ames/Technicon, France).

Determination of Lead

Lead in blood was determined by atomic absorption spectrometry (AAS) as described by the method of Hassel [13]. One mL of 5% triton x-100 was added to 5 mL well mixed EDTA blood to lyse the erythrocytes and release lead. Mixing the content of the tube in a vortex mixer enhanced rapid haemolysis. One mL of 2% ammonium pyrrolidine dithiocarbamate solution was added to chelate the lead. The solution was then extracted with 5 mL methyl-isobutyl ketone and the organic supernatant separated into screw-capped specimen containers and analysed in a Perkin-Elmer 703 Atomic Absorption Spectrophotometer at a wavelength of 283.3 nm.

Determination of Zinc

Zinc in serum was determined by an AAS method of Smith, Butrimovitz and Burdy [14]. Samples were diluted 1 in 5 with deionised water. The instrument was then set at zero with 5% glycerol in deionised water. Diluted samples and standards were then serially aspirated and analyzed at 214 nm using Perkin-Elmer

model 703 Atomic Absorption Spectrophotometer (Perkin-Elmer, Illinois, USA.).

Evaluation of Renal Function

There were no statistically significant differences ($p > 0.05$) observed for sodium, potassium chloride and bicarbonate in exposed subjects compared to controls (unexposed subjects). Occupational lead exposure significantly increased serum uric acid ($p < 0.01$) and inorganic phosphorus ($p < 0.05$), but had no significant effect on serum urea, creatinine and calcium ($p > 0.05$) compared with unexposed subjects (Table 1).

Table 1: Serum urea, creatinine, uric acid, calcium and inorganic phosphorus in human subjects occupationally exposed to lead and in unexposed subjects.

Group	Urea (mmol/L)	Creat (μ mol/L)	Uric acid (μ mol/L)	Calcium (mmol/L)	Inorg.P (mmol/L)
Exposed subjects	5.2 ± 1.2	99 ± 18	$357 \pm 123^*$	2.3 ± 0.2	$1.5 \pm 0.5^*$
Unexposed subjects	4.9 ± 0.6	102 ± 18	$228 \pm 105^*$	2.4 ± 0.2	$1.2 \pm 0.4^*$

All values are means $\pm$ 1 SD, * = statistically significant, n = 25

Evaluation of Liver Function

Occupational lead exposure significantly ($p < 0.05$) increased the activities of alkaline phosphatase, alanine aminotransferase, and aspartate aminotransferase (Table 2). However, no significant differences were found exposed on serum proteins in exposed compared with unexposed subjects.

Table 2: Serum alkaline phosphatase (ALP), alanine aminotransferase (ALT) and aspartate aminotransferase (AST) concentrations in human subjects exposed to lead and in unexposed subjects.

Group	ALP (iu/L)	ALT (iu/L)	AST (iu/L)
Exposed Subjects	$66 \pm 18.9^*$	$11.4 \pm 4.0^*$	$15.8 \pm 4.4^*$
Unexposed Subjects	$78.7 \pm 22.4^*$	$6.8 \pm 2.7^*$	$9.6 \pm 3.5^*$

All values are means $\pm$ SDs, *= statistically significant at $p < 0.05$. n = 25.

Blood Lead and Zinc Levels In Human Subjects

The mean blood lead levels in all occupationally exposed human subjects were significantly ($p < 0.05$) higher compared with unexposed subjects (Table 3). Occupational exposure to lead produced highly significantly decreased ($p < 0.001$) in serum zinc levels (Table 3).

Table 3: Blood lead (BPb) and serum zinc levels in exposed and unexposed subjects.

Group	BPb (μ g/dL)	Serum Zn (μ g/dL)
Exposed Subjects	$59.6 \pm 15.9^*$	$71.3 \pm 14.4^*$
Unexposed Subjects	$35 \pm 7.9^*$	$108.4 \pm 16.9^*$

All values are means $\pm$ SDs, *= statistically significant, at $p < 0.05$. n = 25.

Discussion

The study investigated the effect of lead exposure on selected markers of renal and liver function artisans in mechanic villages in Nnewi, Nigeria. The results showed that occupational lead exposure did not have any effect on electrolytes, urea and creatinine. The kidneys have considerable reserve capacity. This may explain the lack of significant differences in urea and creatinine in the groups studied. Clinical manifestations of renal

impairment do not become evident until more than 50% of the nephrons have been destroyed [15]. These observations may suggest that both urea and creatinine are not sufficiently sensitive to detect renal impairment of lead poisoning.

However, uric acid and phosphate levels were significantly raised compared with controls. Uric acid in man is derived from the breakdown of purines and is essentially the product of the action of the enzyme xanthine oxidase on xanthine and hypoxanthine. Elevated levels of uric acid have been reported as a constant finding in lead toxicity [3]. The mechanism through which lead exposure raises the level of uric acid is unclear, but is thought to be due to damaged renal tubules by lead [3]. Uric acid has been suggested as one of the antioxidants in plasma [16]. Therefore elevations in uric levels in occupationally exposed artisans in this study may be an antioxidant response to lead toxicity.

Phosphate is an intracellular anion whose level in tissues may be altered during cell membrane damage. In the work presented here, phosphate level in the occupationally exposed artisans was significantly higher than in controls. The finding is consistent with that of Papaionnu, Sohler and Pfeiffer [17], who found significant increase in phosphate in lead exposed workers. Lead is known to interfere with cell membrane and may also increase cell breakdown [18]. The increase in phosphate in this study may be due to cell membrane damage as a result of exposure to lead.

Three hepatocellular enzymes and serum albumin were used to evaluate the function of the liver. The liver synthesizes plasma protein, among which is albumin. No significant changes in albumin were observed between occupationally lead exposed artisans and controls. Therefore lead exposure in this study had no effect on the synthetic function of the liver. The activity of alkaline phosphatase was significantly lower in exposed subjects than in controls. Occupational exposure in this study decreased zinc levels. Zinc functions as catalytic, and structural component of zinc-containing enzymes such as alkaline phosphatase [19]. Zinc may also be involved in regulating the amount of enzyme that is synthesized and therefore available for activity [19]. The serum zinc level in the artisans in this study was significantly lower than that found in the control subjects. The decrease in the activity of alkaline phosphatase in this study may be related to the decrease in zinc level in exposed artisans. There has been no previous report of decrease or increase in the activity of alkaline phosphatase in occupationally lead exposed subjects in this environment.

Alanine and aspartate aminotransferase activities were elevated in occupationally exposed artisans than in controls. Similar findings of increased ALT and AST have been reported [20]. Studies by Kapaki, Verelas, Syrigou et al [21], indicate that the activities of alanine aminotransferase and aspartate aminotransferase are

elevated in gas station workers while the activity of alanine aminotransferase is elevated in taxi drivers.

Blood lead levels in occupationally lead exposed artisans were significantly higher than in control subjects. This is similar to previous studies of occupational lead exposure [22]. The mean blood level of 59.6 µg/dL found in the artisans in this study is consistent with the level indicative of severe lead poisoning [23].

The elucidation of the adverse effects of lead [24] has led to the lowering of the formerly acceptable permissible lead levels (PEL) in adults from 40 µg/dL [25] to 20 µg/dL [26]. In this study, all exposed subjects (100%) had blood lead levels above 20 µg/dL, the PEL for blood lead in adults [26]. Twelve subjects (48%) had blood lead levels between 50 µg/dL and 70 µg/dL. Five subjects (20%) had blood lead levels equal to or above 80 µg/dL. Interestingly, twenty two unexposed subjects (88%) had blood lead levels between 25 µg/dL and 48 µg/dL, (with a mean of 35 µg/dL) values known to cause neurobehavioral and intelligence deficits in adults [27]. Only three unexposed subjects (16%) had blood lead levels within the upper permissible lead level. The finding of elevated blood lead levels in both occupationally exposed artisans and control subjects in Nnewi is a serious source of worry and is probably an indication of the widespread nature of lead poisoning in Nigeria. This calls for legislation to reduce the lead content of Nigerian petrol and enforce strict regulation on the siting of industries in designated industrial layouts.

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