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Serum Lead and Micronutrients Levels in Public Transport Drivers in Osogbo, Nigeria

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Authors' contributions

This work was carried out in collaboration between both authors. Authors AA and AKE designed the study and wrote the protocol. Author AA wrote the first draft of the manuscript. Authors AA and AKE performed the statistical analysis. Author AA managed the analyses of the study. Authors AA and AKE managed the literature searches. Both authors read and approved the final manuscript.

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ABSTRACT

Background of Study: Toxic exposure to lead (Pb) in humans occurs through environmental and occupational sources like leaded gasoline. Despite the ban on the use of leaded gasoline by many regulatory authorities due to its adverse health effects, Nigeria remains among nations using leaded fuel.
Aim: The study aimed to determine toxic lead exposure to gasoline fumes and its impact on plasma levels of micronutrients (zinc [Zn], copper [Cu], and calcium [Ca]) in public transport drivers in Nigeria.
Materials and Methods: The case-control study, compared serum micronutrient levels of 40 occupational lead exposed public transport drivers with physically matched 40 non-occupational lead exposed civil servicemen. The concentrations of Pb, Zn, Cu and Ca in the serum of the study subjects were determined by atomic absorption spectrometry.
Results: Results analyzed with the *t*-test showed that mean serum lead level in public transport drivers was significantly (*P*=0.000) high compared to the control group. Also, micronutrient levels

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were significantly (*P*=0.000) lower in occupational than non-occupational lead-exposed men. Pearson correlation analysis results showed significant positive correlation between Cu and Ca (*r*=0.481, *P*=0.002), Zn and Cu (*r*=0.635, *P*=0.000), Zn and Ca (*r*=0.456, *P*=0.003). Whereas an inverse but non-significant correlation between lead and Cu (*r*=-0.275), lead and Ca (*r*=-0.130), lead and Zn (*r*=-0.121) was observed at the 0.05 level.

Conclusion: In conclusion, the study results indicate that exposure to lead can significantly decrease serum zinc, copper, and calcium. The decrease in micronutrient level in the drivers could be through renal loss, mediated by the nephrotoxic effect of lead.

Keywords: Gasoline; lead; zinc; calcium; copper.

1. INTRODUCTION

Gasoline or petrol is a transparent, volatile and highly flammable extract of crude oil composed mainly of aliphatic hydrocarbons. It is used as fuel in internal combustion engines of automobiles and power plants [1]. The characteristic of a gasoline blend to resist igniting too early (causing knocking) is measured by its octane rating. Additives are thus introduced into gasoline to increase its octane rating for better performance, reduction in fume emission and prevention of engine knocking [1]. Tetraethyl lead is among the anti-knocking agents added to enhance the performance of gasoline.

The attendant health effects associated with environmental lead has been previously described as a mistake of the twentieth century [2]. This negative effect informed the worldwide policy of leaded gasoline phase-out [3]. Yet gasoline used in some nations of the world like Nigeria contains toxic levels of lead in contrast to their developed counterparts [4]. The national conference on the phase-out of leaded gasoline in Nigeria (NCPLGN), planned to reduce the lead content of Nigerian gasoline from 0.74 to 0.15 g/L by the year 2002 and the total elimination of leaded gasoline by 2004. Leaded gasoline phase out in Nigeria was to be achieved through the nation's only government-owned refineries [5]. Due to the inaction of the Nigerian government, environmental regulation and control policies are weak. There is no evidence of leaded gasoline phase-out in the country and no new strategic plan of tackling the menace. Nigeria, till date depends heavily on imported gasoline to power internal combustion engines, increasing the chances of importing leaded gasoline. A large majority of automobiles on Nigerian roads are old-model, already used vehicles built to run on leaded gasoline, imported from developed nations. New modeled vehicles designed to use lead-free gasoline have wasted their catalytic converters with leaded gasoline. Due to the poor economy of the nation, many Nigerian vehicles lack maintenance and are driven without air condition systems on poorly maintained roads [4]. Consequently, a large number of vehicles emit fumes composed of unburnt hydrocarbons and lead into the atmosphere, polluting the air [6].

Lead is the most important environmentally toxic heavy element [7]. It is widely and abundantly distributed in the world [8]. Human exposure to lead and its compounds occur through occupational and environmental sources like leaded gasoline, water, leaded paints, pottery, battery recycling and printing works [9]. The main source of human exposure is air and 90% linked with leaded gasoline [5]. Lead is a highly toxic metal affecting almost every organ in the body, with ingestion, inhalation and dermal contact as routes of body exposure [7]. There are reports in many countries determining community exposure to environmental toxic lead and its impact on health [10]. Although there are no national surveys of blood lead levels in the general Nigerian population. Previous studies reported by other authors indicate very high blood levels of lead [11-14] compared with acceptable serum (<0.035 µg/dl [<3.5 µg/dal]) or blood (<10 µg/dl[<1000 µg/dal]) limits [15]. The United States department of health and human services recommends maintenance of less than 10 µg /dL blood lead levels (BLL) for adults [16]. However, based on research reports of adverse health effects even at lower levels, the Centers for Disease Control and Prevention (CDC)'s Adult Blood Lead Epidemiology and Surveillance (ABLES) program, recommends adult BLLs of >5µg/dL [16,17]. Toxic lead levels are associated with pathological conditions; anemia, nephropathy, neuropathy, infertility, and immunosuppression [7].

Micronutrients are essential nutrients required by the body in trace amounts or tiny quantities in the order of less than 100 mg/day for proper body

functioning. Micronutrients are composed of four major classes: macro elements, trace elements, vitamins, and organic acids [18]. Macro elements have multiple roles within the body. They work together with vitamins to initiate hormone production and enhancement of metabolic processes. Trace elements participate in tissue, cellular and sub-cellular functions; these include immune regulation by humoral and cellular mechanisms, nerve conductions, muscle contractions, membrane potential regulations, mitochondrial activity, and enzyme reactions. Trace elements interact with vitamins and macro elements to enhance their effects on the body. They are accepted as essential for human health and with diverse metabolic functions [19]. Lead has been shown to mimic the action of some trace elements, by competitively binding to the active sites of their respective enzymes as co-factors thereby inactivating such enzymes [20]. Trace element deficiencies are associated with inadequate dietary intake or may result from metabolic imbalances due to antagonistic and synergistic metal interactions [21].

This present study determined serum lead, calcium, zinc, and copper in professional drivers exposed to gasoline fumes. There is available literature for elevated blood lead levels in public transport drivers [22]. However, no study exists pertaining to the impact of lead toxicity on micronutrients in this occupational lead exposed group. The aim of this study is to determine the impact of lead on plasma levels of micronutrients (Zn, Cu, and Ca) in public transport drivers in Nigeria.

2. MATERIALS AND METHODS

2.1 Study Area and Population

The occupational health study was conducted at the Ladoke Akintola University of Technology Teaching Hospital, Osogbo, Nigeria. The test group comprised of male public transport drivers aged 25-55 years, who were members of the National Union of Road Transport Workers, Osogbo unit, Nigeria. While the control group was apparently healthy civil servicemen of the same age range.

2.2 Study Design

The case-control study was approved by the ethical committee of the teaching hospital. Serum lead and micronutrient levels of 40 male public transport drivers were compared with 40 age and BMI matched male controls.

2.3 Sample Size and Technique

The sample size was determined using the formula for case-control studies that compare two group means [23]; $n=1+2C(s/d)^2$, where s is the standard deviation (an estimate of the population standard deviation of serum lead), d is the effect size (the estimated mean difference of toxic and non-toxic serum lead levels) and C is a constant dependent on the level of significance and statistical power. At a significance level of 5%, and statistical power of 90%, C is 10.51. The calculated sample size was 33.8, which was approximated to 40 for each group. In total, 80 participants were randomly drawn from public transport drivers and civil service men.

2.4 Selection Criteria

A written informed consent was sought from the individual participants by educating them on the need and relevance of the study. Information regarding their bio-data, lifestyle, occupation/career, the condition of the vehicle driven and medical history were obtained.

2.4.1 Inclusion criteria

Public transport drivers: Drivers of non-air conditioned vehicles that consistently drove at least three times a week and occupationally exposed to gasoline fumes for a period of 5 to 20 years. Civil servicemen: Those exposed to lead only environmentally with no history of occupational exposure to lead.

2.4.2 Exclusion criteria

The exclusion criteria comprised of participants placed on medications that could interfere with micronutrient level during sample analysis. Also, participants with medical conditions that may interfere with micronutrient status in the body were excluded. Participants who refused to give consent were voluntarily allowed to withdraw from the study.

2.5 Data Collection

Body weight to the nearest 0.1 kg and height to the nearest centimeter were measured with the subjects barefooted and in light clothing. Body mass index (BMI) was calculated as weight (kilograms)/height (meters squared). By venipuncture, 5 ml of blood was collected into plain tubes to obtain serum, after allowing the clot to retract with subsequent centrifugation for

15 minutes at 4000 rpm. The extracted serum was stored at -20ºC until the time of analysis.

2.5.1 Laboratory methods

The concentrations of Pb, Zn, Ca and Cu in serum were determined by atomic absorption spectrometry-AAS method [24]. Specifically, serum Zn, Ca and Cu were determined with flame-AAS and Pb with graphite furnace-AAS. The atomic absorption spectrometry method is based on the principle that; when monochromatic light emitted from specific metal cathode lamp, is passed through an atomized sample, a low energy level is created, enabling the atoms to absorb light of unique wavelength. The amount of light absorbed is proportional to the concentration of the metal in the sample. Lead (Pb), Zn, Ca and Cu hollow cathode lamps were operated under standard conditions. Grade concentrated acids (hydrochloric and nitric acid) were diluted with ultrapure water and used as diluents for serum, standards, and controls. The serum was centrifuged and supernatant separated into clean element free polypropylene tubes. The supernatant for Pb determination was further modified to enhance its sensitivity during analysis. Standard solutions were prepared by the dilution of 1,000 µg/ml Pb, Zn, Ca, and Cu stock standard solutions**.** Working standards of 0.1, 0.3, 0.6, 0.9, 1.2 µg/l for Lead; 0.2, 0.4, 0.6, 0.8, 1.0 µg/ml for copper; 0.2, 0.4, 0.6, 0.8, 1.0 µg/ml for zinc; and 1.0, 2.0, 3.0, 4.0, 5.0 µg/ml for calcium were obtained and the concentration values used as the ranges for calibration graphs. Glycerol was added to the blank (sample diluent) and standard to match the viscosity of the serum. The blank solution was introduced into the atomizer and absorbance of the spectrometer set at zero. The working standards were sequentially introduced from the most diluted to the most concentrated, absorbances were obtained and calibration curves plotted. The sample solutions were also introduced into the atomizer, their absorbances read and used in determining metal concentrations from the calibration curves.

Contamination prevention was achieved by using chemicals/reagents of analytical grade. Ultrapure reagents and deionized water were used throughout the analysis. Analytical containers were properly washed with nonionic detergents, rinsed severally with deionized water, and dried in the oven for 24 hours. Duplicate measurements were performed during standard/sample measurement, and the mean of both measurements used as the final value. Checking for accuracy and precision of the AAS methods, samples were spiked with a standard of known concentration. The following coefficients of variation were obtained: 5% and 4% at 0.1 and 1.0 µg/l of lead respectively; 6.5% and 4% for copper, 7% and 4.5% for zinc, 6% and 5% for calcium at 0.1 and 1.0 µg/ml, respectively. The method adopted in this study was sensitive and suitable for detection at low levels. Detection limits were 0.05 µg/l for lead, 0.05 µg/ml for copper, 0.01 µg/ml for zinc, and 0.08 µg/ml for calcium. the calibration curve was linear from the start point up to 1.2 µg/l for Pb, 0.9 µg/ml for Cu, 0.9 µg/ml for Zn, and 2.8 µg/ml for Ca. In each analysis batch, one sample of the certified reference material, Seronorm^{TM} Trace elements serum Level 1 (SERO201405) and two samples of one healthy volunteer serum specimen were analyzed for the validation of the analytical methods.

2.6 Statistical Analysis

Statistical analysis was performed using SPSS version 21 of IBM Armonk, New York, United States. Descriptive statistics (mean and standard deviation) was used in expressing the concentration of metals in the groups compared. Student's *t*-test was used in comparing the means of parameters in public transport drivers and civil servicemen. Pearson correlation analyses were done to determine the association between metals measured in public transport drivers. Two-tailed $P < 0.05$ was considered statistically significant.

3. RESULTS

Serum lead level in public transport drivers shown in Table 1 was significantly (P=0.000) high compared to anthropometrically matched civil servicemen. Comparing micronutrient levels between the study groups showed that Cu, Ca, Zn were significantly lower (P=0.000) in occupational than non-occupational lead-exposed men (Table 1, Fig. 1).

Pearson correlation analysis results presented in Table 2, showed significant positive correlation between Cu and Ca (r=0.481, P=0.002), Zn and Cu (r=0.635, P=0.000), Zn and Ca (r=0.456, P=0.003). Table 2 also revealed an inverse correlation between lead and Cu (r=-0.275), lead and Ca (r=-0.130), lead and Zn (r=-0.121) however, non-significant at the 0.05 level.

Table 1. Anthropometry, micronutrients, lead in public transport drivers and civil servicemen

Parameters	Drivers n = 40	Civil servicemen n = 40	t-value	p-value
BMI (Kg/m^2)	24.15±3.30	24.63±3.44	0.58	0.539
Age (years)	38.52±10.80	38.20±10.37	0.11	0.901
Copper (µg/dl)	75.00±19.70	83.60±15.80	4.8	0.000*
Calcium (µg/ml)	80.10±16.06	88.32±12.04	8.8	0.000*
Zinc (µg/dl)	74.30±16.20	86.30±13.10	13.3	0.000*
Lead (µg/dal)	11.00±5.00	7.00±5.00	26.2	0.000*

*mean±standard deviation, n-number of subjects, *significant, BMI-body mass index*

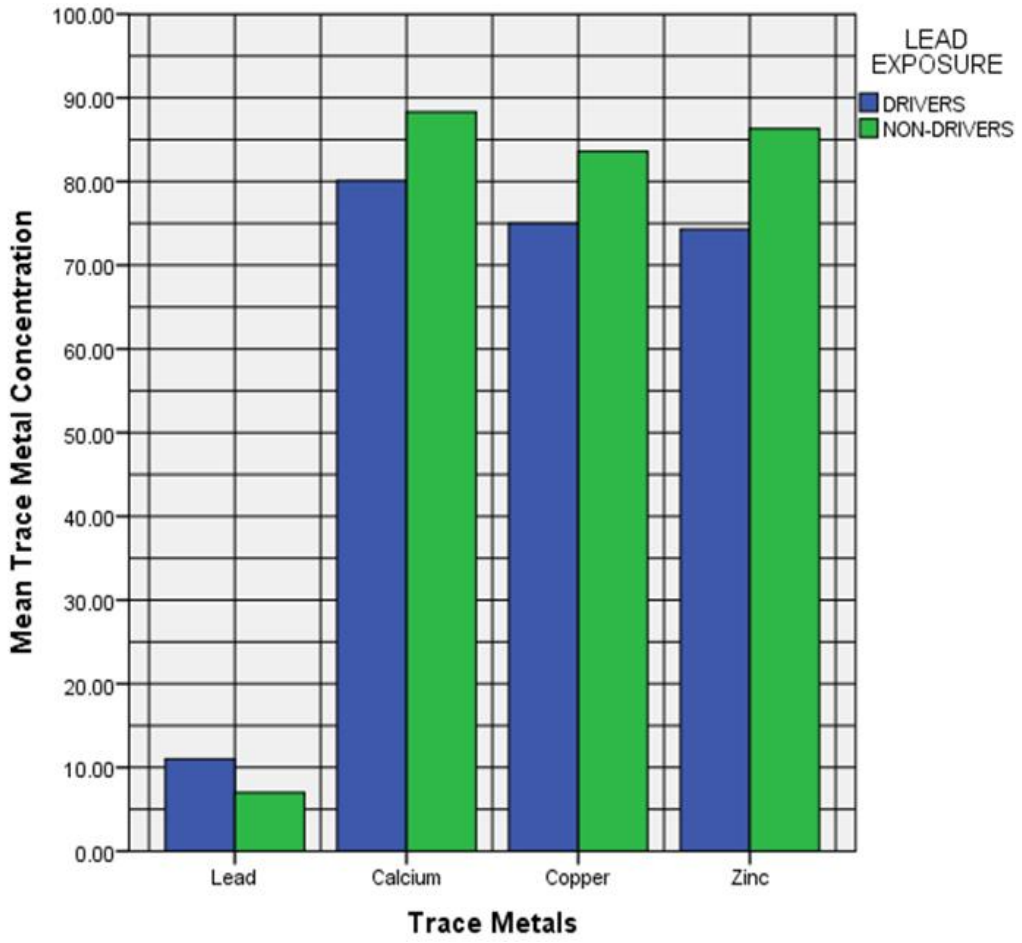

Fig. 1. Comparison of lead, calcium, copper, zinc in public transport drivers and civil servicemen

Table 2. Correlation amongst micronutrients and lead in public transport drivers

R	Copper	Calcium	Zinc	Lead
Copper	1.000	0.481**	0.635**	-0.275
Calcium	0.481**	1.000	0.456**	-0.130
Zinc	0.635**	0.456**	1.000	0.121
Lead	-0.275	-0.130	-0.121	1.000

**Correlation (r) is significant at the 0.05 level, **Correlation is significant at the 0.01 level*

4. DISCUSSION

Directives on the reduction of lead in gasoline have been issued in Nigeria. However, non-compliance with these directives persists, as leaded gasoline continue to thrive in the Nigerian petroleum industry. It is hoped that our study will provide knowledge about the effect of lead exposure on public transport drivers who are frequently exposed to gasoline fumes. The adverse effects of lead on the body systems at both high and low doses are well documented. Lead is quickly absorbed into the bloodstream and is shown to have adverse effects on certain organ systems like the central nervous system, the cardiovascular system, kidneys, and the immune system [7]. The impact of micronutrient status on lead toxicity has been earlier reported by other studies [25]. However, investigations into the impact of lead toxicity on micronutrients are vague. Our study sought to find the effect of this toxic metal on micronutrients (zinc, copper, and calcium) levels.

Our study revealed elevated serum lead levels in public transport drivers compared to civil service men. This finding is consistent with the study of Sawas and Eldeib who observed elevated levels of serum lead in public transport drivers compared to civil servicemen [26]. Saliu et al., Dioka et al., Alasia et al., Onuegbu et al. found elevated blood lead levels in occupationally exposed automobile mechanics and petrol attendants in Nigeria [11,27,28,29].

Our study observed reduced serum micronutrient (Ca, Zn, Cu) levels in public transport drivers compared to civil servicemen. Also, a negative but no statistical correlation existed between lead and micronutrients studied. The study further observed a positive correlation of Cu with Zn, Ca with Cu, and Zn with Ca which was statistical. A study similar to this present study, however in battery workers showed decreased serum zinc, calcium and copper levels compared to non-occupational lead-exposed men [30]. In a population of roadside gasoline dispensers, Adamu et al. found low plasma levels of Zn and Cu compared to non-occupationally exposed Nigerians [31]. In children, low dietary intake of zinc, copper, and calcium has been associated with increased blood lead levels [32]. Ahamed and Siddiqui showed that the mean blood levels of zinc and calcium were significantly lower in anemic children with blood lead levels ≥10 µg/dl than those with blood lead levels <10 µg/dl [33] They further observed decreased blood calcium and zinc levels with increasing blood lead levels in the anemic children studied [33]. Our observation of an inverse correlation of Zn and Ca with lead, corroborates with the findings of Mogwasi et al. [34]. Ugwuja et al., showed decreased plasma zinc level in Nigerian pregnant women with high blood lead levels compared to non pregnant women with low blood lead levels [35]. The concentrations of Cu and Zn are known to correlate and interact in the body [36]. Correlation between serum levels of Zn and Cu in gasoline filling station workers has been reported by the study of Mahmood [37]. After controlling for gender and age, a positive correlation between total blood Ca and Zn was observed by the study of Ji et al. [38].

The impact of toxic lead on micronutrient status observed in our study could be mediated via renal and gastrointestinal mechanisms. Experimental evidence of impaired and altered nephritic function by lead has been reported [39]. Early exposure to lead can cause renal proximal tubular dysfunction, chronic interstitial nephritis and eventually irreversible progressive chronic kidney disease that culminate in end-stage renal failure [40]. Lead has been shown by studies to stimulate urinary excretion of zinc and copper, interfering with their reabsorption in kidney and inhibition of ceruloplasmin activity in plasma [41]. Evidence also exists for interactions between lead and micronutrients at the level of intestinal absorption, metabolism and sites of action in the body [25]. Lead is shown to change the gastrointestinal absorption and tissue concentrations of micronutrients, such as Zn, Cu, and Ca [25]. The deficiency of zinc, copper, and calcium has been shown to increase the gastrointestinal absorption and toxicity of lead [42]. Vice versa, Pb competes and impairs the gastrointestinal absorption of micronutrients [25]. Calcium deficiency state mobilizes lead from the bone and distributes it to blood and soft tissue [43]. It appears likely that Pb may contribute to the development of adverse health effects in humans through mechanisms involving its interference with the metabolism of micronutrients.

The use of small sample size, measurement of relatively few micronutrients and determination of micronutrients in only one sample type are limitations of this study. We recommend a follow-up study encompassing a treatment plan and the determination of more micronutrients in other body samples (especially urine and faeces).

5. CONCLUSION

In conclusion, the study results indicate that exposure to lead can significantly decrease serum zinc, copper, and calcium. The decrease in micronutrient level in the drivers could be through renal and gastrointestinal loss, mediated by the tissue effect of toxic lead. Our study is relevant when evaluating possible mechanisms of the impact of lead toxicity on micronutrient status.

CONSENT

As per international standard or university standard, patient's written consent has been collected and preserved by the authors.

ETHICAL APPROVAL

As per international standard or university standard, written approval of Ethics committee has been collected and preserved by the authors.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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