Original Research Article

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

ABSTRACT

Background of study: Toxic exposure to lead (Pb) in humans occurs through environmental and occupational sources like leaded gasoline. Despite the ban of 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 spectrophotometry. **Results:** Results analyzed with the *t*-test showed that mean serum lead level in public transport drivers was significantly (P=0.000) higher than the control group. Also, micronutrient levels 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). Where as an inverse but non-significant correlation between lead and Cu (r=-0.275), Ca (r=-0.130), 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 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 [2]. One of the additives to gasoline that significantly increases its octane rating and anti-knocking potential is tetraethyl lead. The environmental pollution and attendant health effects associated with tetraethyl lead, has been described as the mistake of the twentieth century [3.] This negative effect, led to its ban as an additive to gasoline by regulatory authorities [4]. Yet gasoline used in some nations of the world like Nigeria contains high lead levels in contrast to most developed nations [5]. 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 0.15g/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 nations only government owned refineries [6]. However, no evidence to show that the program has been implemented. Unfortunately, Nigeria for decades 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 modeled already used vehicles built to run on lead gasoline, imported from European nations. New modeled vehicles designed to use lead-free gasoline have wasted their catalytic converters by burning leaded gasoline. Due to the poor economy of the nation, many vehicles lack maintenance and are driven without air condition systems on poorly maintained roads [5]. Consequently, a large number of Nigerian vehicles emit blue plumes of bad odor and unburnt hydrocarbons [7] that release a high percentage of gasoline lead into the atmosphere, subsequently inhaled by road users [8].

Lead is the most important environmentally toxic heavy element. It is widely and abundantly distributed in the world [9]. Human exposure to lead and its compounds occurs through occupational and environmental sources like leaded gasoline, water, leaded paints, pottery, battery recycling and printing works [10]. The major source of human lead accumulation in developing countries was found to be airborne lead, 90% of which came from leaded gasoline [6]. Lead is a highly poisonous metal affecting almost every organ in the body, with ingestion, inhalation and dermal contact as routes of body exposure. There are reports in many countries determining community exposure to environmental toxic lead and its impact on health [11]. Although there are no national surveys of blood lead levels in the general Nigerian population. Isolated studies indicate very high blood levels of lead [12-14] compared with acceptable serum (<0.035µg/dl [<3.5 µg/dal]) or blood (<10µg/dl[<1000 µg/dal]) limits [15]. Toxic lead levels have consistently been associated with various pathological conditions like anemia, neurobehavioral deficits, renal impairment, reproductive abnormalities and suppressed immune system [6].

Micronutrients are essential nutrients required by the body in trace amounts or tiny quantities on a day to day basis (generally less than 100mg/day) for proper body functioning. Micronutrients are composed of four major classes: macro elements, trace elements, vitamins, and organic acids [16]. 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 have diverse metabolic characteristics and functions [17]. 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 [18]. Trace mineral deficiencies usually occur when dietary intake is inadequate or result from metabolic imbalances produced by antagonistic or synergistic interactions among metal [19].

Our study measured serum lead, calcium (macro element), zinc and copper (trace elements) in drivers regularly exposed to gasoline fumes. There is available literature for elevated blood lead levels in public transport drivers [20], no study exist pertaining to the impact of lead toxicity on micronutrients in this occupational lead exposed group. Thus, the aim our study is to determine toxic exposure to lead gasoline fumes and its impact on plasma levels of micronutrients (Zn, Cu, and Ca) in public transport drivers in Nigeria.

2. Materials and Method

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 were 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 formular for case-control studies that compares two group means [21]; $n=1+2C(s/d)^2$, 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 biodata, lifestyle, occupation/career, condition of vehicle driven and medical history were obtained.

2.4.1 Inclusion criteria

Public transport drivers: Drivers of non-air conditioned vehicles at least three times a week and occupationally exposed to gasoline fumes for 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 barefoot and in light clothing and 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 clot to retract with subsequent centrifugation for 15 minutes at 4000rpm. The extracted serum was stored at -20˚C until time of analysis.

2.5.1 Laboratory Methods

The concentrations of Lead (Pb), Zn, Ca and Cu in serum were determined by atomic absorption spectrophotometer using an acetylene-air flame [22]. When monochromatic light emitted from a metal (Pb, Zn, Ca, Cu) hollow cathode lamp, is passed through an air-acetylene flame containing dissociated metal atoms in sample. A low energy level is created, that enables the atoms absorbs light of unique wavelength. The amount of light absorbed is proportional to the concentration of the metal in the sample, determined by comparing unknown samples with known standard. The Samples were prepared by adding 1 ml of serum to 3ml of 2NHCl and centrifuged. The supernatant was separated into polypropylene tubes and mixed well. Glycerol was added to the aqueous blank and standard to match the viscosity of the diluted serum. The blank solution was aspirated into the flame and used to set the baseline to read zero absorbance. Metal working calibrators were aspired sequentially from the most diluted to the most concentrated. The resulting absorbances were used to establish calibration curves. The specimens were aspirated and their absorbance's with metal concentrations determined using the calibration curves.

2.6 Statistical Analysis

Statistical analysis was performed using SPSS version 21 of IBM Armonk, New York, United States. The statistical methods included the mean and standard deviation. Student's *t*-test was used in comparing the means of parameters in public transport drivers and civil servicemen groups. Pearson correlation analyses was done to determine the association between metals measured in the 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, Figure 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), Ca (*r*=-0.130), 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*

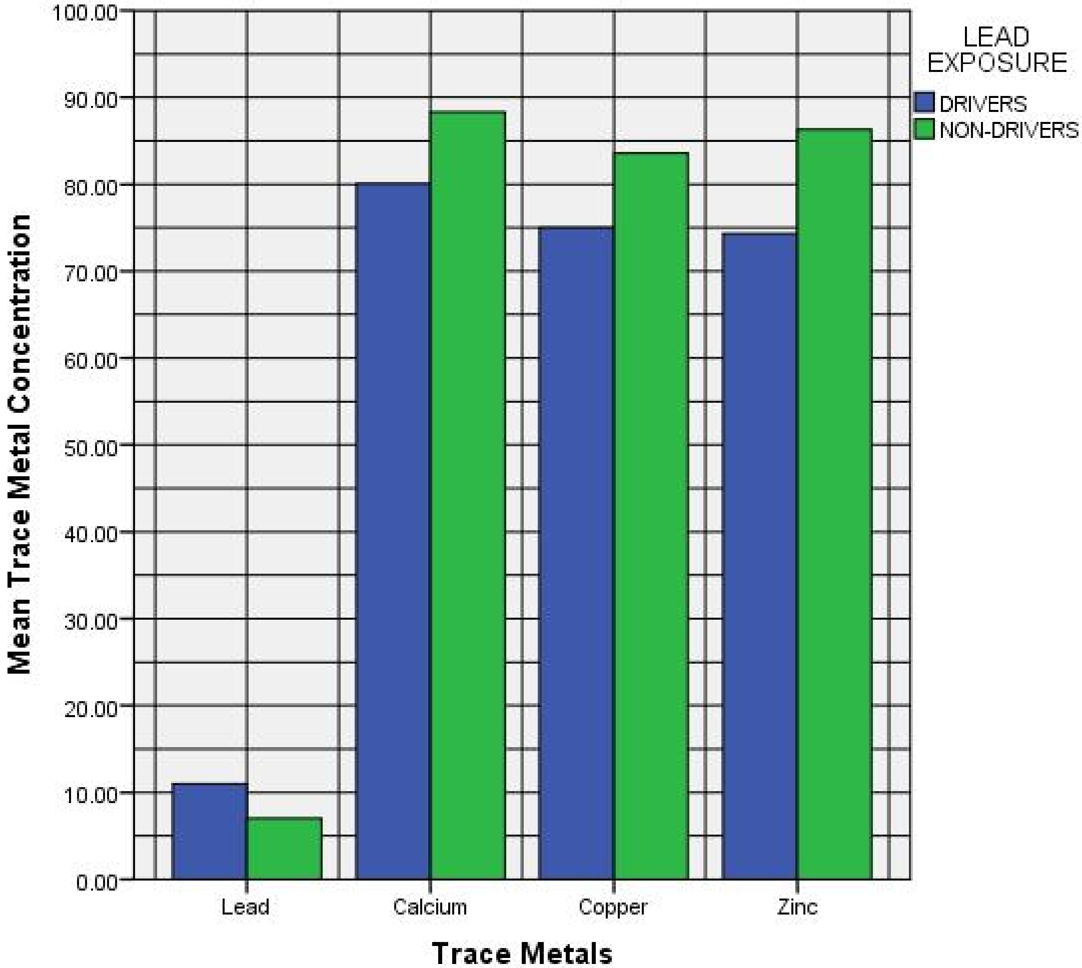

Fig. 1: Comparison of Lead, Calcium, Copper, Zinc in public transport drivers and civil servicemen

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 reduction of lead in gasoline have been issued in Nigeria. However non - compliance to 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 in the blood stream and is shown to have adverse effects on certain organ systems like the central nervous system, the cardiovascular system, kidneys, and the immune system [23]. The impact of micronutrient status on lead toxicity has been reported [24,25]. However, investigations into the impact of lead toxicity on micronutrients is 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 toxic levels of lead (serum≥3.5 µg/dal) in public transport drivers compared to civil servicemen. [26] Dioka et al., observed raised blood lead levels in some Nigerian artisans who were occupationally exposed to lead [27].

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 ours; however in battery workers showed decreased serum zinc, calcium and copper levels compared to non-occupational lead exposed men [28]. In children, low dietary intake of zinc, copper, and calcium has been associated with increased blood lead levels [19]. 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 [29] They further observed decreased blood calcium and zinc levels with increasing blood lead levels in the anemic children studied [29]. In rats, very high dietary levels of lead have been shown to reduce copper ceruplasmin levels [30]. A decreased dietary copper level associated with increased erythrocyte lead concentrations was also observed [30]. Our observation of an inverse correlation of micronutrients with lead, corroborates with the findings of Needleman [18]. 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 [31]. The concentrations of Cu and Zn are known to correlate and interact in the body [32]. Correlation between serum levels of Zn and Cu in gasoline filling station workers has been reported by the study of Mahmood [33]. After controlling for gender and age, a positive correlation between total blood Ca and Zn was observed by the study of Ji et al. [34] Blood calcium concentrations and calcium intake have been inversely related to blood lead levels in both normal and lead-burdened children [35,36]. Calcium deficiency mobilizes lead from the bone and distributes it to blood and soft tissue [35].

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 [37]. 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 [38]. 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 [39]. Evidence also exists for interactions between lead and micronutrients at the level of intestinal absorption, metabolism and sites of action in the body [40,25,24]. Lead is shown to change the gastrointestinal absorption and tissue concentrations of micronutrients, such as Zn, Cu, and Ca [41]. The deficiency of zinc, copper, and calcium has been shown to increase the gastrointestinal absorption and toxicity of lead [42,29]. Vice versa, previous findings by Emsley et al., showed that toxic blood levels of lead, reduced the gastrointestinal absorption of zinc in elderly Chinese

[43]. It appears likely that lead may contribute to the development of adverse health effects in humans through mechanisms involving its interference with the metabolism of micronutrients.

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.

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