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EVALUATION OF THE EFFECT OF GASOLINE ON SERUM CALCIUM, MAGNESIUM AND PHOSPHORUS LEVEL IN PETROL STATION ATTENDANTS IN YENAGOA, NIGERIA

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ABSTRACT

Exposure to hazardous substances like benzene, toluene, xylene, and ethyl benzene (BTEX) present in crude oil portends serious health problem to humans. This present study investigated the effects of petrol fumes on calcium, magnesium and phosphorus levels in serum of petrol station attendants in Yenagoa, Nigeria. A total of fifty (50) apparently healthy male and female subjects within the age range of eighteen (18) and forty (40) years were recruited for this study. Thirty (30) subjects were petrol station attendants; while twenty (20) were non petrol station attendants who served as control. Blood samples were collected from the subjects; the three parameters calcium, magnesium and phosphorus were analyzed spectrophotometrically using 0-cresolpthalein Complexon, Xyldyl blue and Molybdate UV methods respectively. Results showed statistically significant ($P<0.05$) reduction in calcium (2.40±0.42) among exposed subjects compared with the control (2.62±0.19), While Magnesium (0.83±0.08) and phosphorus (1.13±0.17) showed no statistically significant ($P>0.05$) reduction as compared with the control (0.86±0.09 and 1.14±0.22 respectively). Furthermore, there was a statistically significant ($P<0.05$) reduction in calcium (2.13±0.13) and magnesium (0.77±0.65) among subjects ≥ 5 years duration of exposure compared with < 2 years (2.63±0.25 and 0.89±0.03 respectively). Calcium and phosphorus showed statistically significant ($P<0.05$) difference in male and female petrol attendants compared with the control, while magnesium did not. Complications usually associated with hypo or hyper states of these microelements are of serious medical concern.

Thus, the need for intermittent evaluation of these elements among petrol station attendants with ≥ 5 years exposure is strongly advocated.

KEYWORDS: Calcium, Gasoline, Magnesium, Phosphorus, and Petrol Station Attendants

INTRODUCTION

Petroleum is a complex mixture of aliphatic and aromatic hydrocarbons derived from blending fractions of crude oil with brand-specific additives. It contains oxidizing agents such as benzenes, toluene, ethylbenzene and xylene (BTEX), in addition to heavy metals as lead, cadmium, chromium and mercury.[1] These compounds are converted into free radicals or activated metabolites during their oxidation in the liver cells.[2] The actual composition of petrol varies according to the source of crude oil, the manufacturing process and batches.[3]

Petrol fumes are abundant in our environment and the common sources of exposure are refineries, motor parks, oil fields, petrol stations and homes.[4] Petrol station workers are routinely exposed to the hazardous chemicals in petrol fumes via cutaneous and respiratory routes.[5] Long term exposure of petrol attendant to petrol fumes and its harmful products have been reported to cause many health hazards. Among these health hazards are nervous system damage, blood disorders (including anaemia, leukaemia), nephrotoxicity, hepatotoxicity, cardiotoxicity and intoxication leading to serious psychotic problems, anaesthetic effects, dermatitis, cancer and musculoskeletal damages.[2,6,7] Common symptoms reported by petrol station attendants as documented in different studies include chronic cough, breathlessness, nausea, vomiting, redness of the eyes, musculoskeletal disorders, low back pain, headache, fatigue and dizziness.[7,8]

Several factors are known to modulate the distribution of micronutrients such as calcium, sodium, potassium, chloride, magnesium, and phosphorus in the human body. These factors may include; environmental, or genetic factor. Moreover, occupational exposure to certain chemicals, changes in life style and dietary habits have been known to affect the concentration of micronutrients in human. A previous study by Awodele et al.[9] reported that exposure to petrol fumes interfere with absorption of some micronutrients such as potassium (K+), sodium (Na+), calcium (Ca2+), Magnesium (Mg2+), phosphorus (p), chloride (Cl–), and bicarbonate (HCO3–). Some of these micronutrients are known to act as co-factors and co-enzymes, while others are involved in preventing free radical induced damages,

preventing the formation of reactive oxygen species, scavenging them or promoting free radical decomposition in the body.[10]

Calcium is known to play major role in skeletal mineralization, as well as a wide range of biological functions among which include muscle contraction, oocyte activation, building strong bones and teeth, blood clotting, nerve impulse, transmission, regulating heart beat and fluid balance within cells.[11] The requirement of calcium in the human body is mainly dependent on the state of its metabolism, which is in turn regulated by several mechanisms such as intestinal absorption, renal absorption and bone turnover.[10] Certain hormones like Parathyroid Hormone (PTH), 1,25 –dihydroxyvitamins D (1,25(OH), ionized calcium itself and their corresponding receptors in the gut, kidney and bone are major players involved in the interplay of calcium biochemistry. A large amount of total calcium is present in the skeleton as calcium – phosphate complexes, primarily as hydroxyapatite which is responsible for its material properties of bones.[12]

Phosphorus is an essential element of cellular membrane lipid bilayer (phospholipids) and ultracellular compounds like nucleic acid and nucleoprotein.[13] Most ultracellular phosphates exist as organic phosphates in creatinine phosphate, adenine triphosphate (ATP) and 2,3 - diphosphogycerates. Bone contains about 85% of the body's phosphate. The rest is located primarily inside cells, where it is involved in energy production.[10] Raina *et al*.[14] have reported that changes in serum phosphate level do not necessarily reflect the body's total store of phosphorus.

Magnesium is an important element in biological system, and plays a vital role in over 300 enzymatic reactions in the human body.[10] Its numerous functions include helping with muscle and nerve function, regulating blood pressure, and supporting the immune system.[15] Thus, its inadequate intake frequently causes muscle spasm which is associated with cardiovascular diseases, blood pressure, anxiety disorder, osteoporosis and cerebral infarction.[10]

Imbalances of micronutrients such as calcium, phosphorus and magnesium result in a number of serious clinical complications including myocardial failure, respiratory failure, proximal myopathy, increased osteolysis and excessive formation of uncalcified osteoid leading to osteomalacia, rickets, osteoporosis and fractures.[16] Many studies worldwide have been documented on the health effects of the petroleum products on the petrol station attendants.

However, there is paucity of data on the effects of gasoline on serum calcium, magnesium and phosphorus level in petrol station attendants. Therefore, this study was undertaken to evaluate the effect of gasoline on serum calcium, magnesium and phosphorus in petrol station attendants in Yenagoa, Nigeria.

MATERIALS AND METHODS

The study was carried out in Yenagoa, Bayelsa State, Nigeria. Four identified Petrol stations that sell petrol product: NNPC Petrol station, Don-Cont petrol station, Kemie-owei Petrol station, and Jovero petrol station were used. A total of fifty (50) subjects comprising 30 males and 20 females between the ages of 18-35 years were recruited for the study. Thirty (30) subjects were petrol station workers who had worked for about one (1) to ten (10) years. The remaining twenty (20) subjects were unexposed individuals who served as control. Subjects who consented to the study and without a medical history of any known metabolic disorder, bone disease and non-smokers were included for the study. Subjects who do not consent to the study and with a medical history of known metabolic disorder and bone disease were excluded from the study. Also excluded are cigarette smokers. Ethical issues were handled and approval was obtained from institutional authorities concerned. Also, the individual consent of the petrol attendants was obtained before specimen collection.

Blood specimen collection

Blood samples were collected from Fifty (50) subjects. Venous blood was collected under septic condition using venopuncture techniques**.** Blood sample of 5ml were collected from subject with sterile syringe from antecubital vein and dispensed into a clean plain bottle. The blood was allowed to clot properly, and clot dislodged. The samples were centrifuged at 3000rpm for 5minutes and the supernatant serum separated and transferred into a labelled plain bottle with the aid of micropipette. The serum sample was stored at -20^{0}C and all analysis were carried out within 24 hours of serum sample collection.

Analytical methods

Calcium: Calcium was measured according to 0-cresolpthalein complexon (CPC) method as described by Kessler and Wolfman[17] using Aggape reagent. The principle is based on the reaction of calcium in the alkaline solution to form an intense violet coloured complex which gives a maximum absorbance at 578nm wavelength. Procedure: Three test tubes were labeled “Blank”, “Standard”, “Test” respectively. 1000ul of working reagent was pipetted into each tube. 25ul sample and standard was added to respective test tubes. The tubes were mixed and

allowed to incubate for 5 minutes at room temperature. Reading of absorbance was taken at 570 nm against reagent blank.

Magnesium: Magnesium was measured according to Xyldyl blue method as described by Natelson[18] using Aggape reagent. The principle is based on the fact that magnesium in serum sample react with xyldyl blue to form coloured compound in alkaline solution which was measured at 546nm wavelength. Procedure: Three test tubes were labeled "Blank", "Standard", "Test" respectively. 1000 ul of working reagent was pipetted into each tube. 10ul sample and standard was added to respective test tubes. The tubes were mixed and allowed to incubate at 37°C for 3 minutes. After incubation, the spectrophotometer was zeroed with the reagent blank at 546 nm. Reading of absorbances of all tubes were taken.

Phosphorus: Phosphorus concentration was measured according to molybdate UV method as described by Tietz,[19] using Aggape reagent. The principle is that when ammonium molybdate react with sulphuric acid in the presence of phosphorus, a complex called phosphomolybdic complex which was measured at 340nm. Procedure: Three test tubes were labeled "Blank", "Standard", "Test" respectively. 1000 ul of working reagent was pipetted into each tube. 10ul sample and standard was added to respective test tubes. The tubes were mixed and allowed to incubate at 37°C for 5 minutes. Absorbance of all tubes were taken at 340nm.

Statistical analysis

Statistical analysis was done by descriptive and inferential statistics using chi-square test and one-way ANOVA. The concentrations obtained was expressed as mean ± standard deviation. The software used for the analysis were SPSS (statistical package for Social Science) version 23.0. The means were separated and compared at 0.05 level of significance.

RESULT

The result revealed a statistically significant ($P<0.02$) decrease in the mean value of calcium (2.40±0.42) in petrol station attendants when compared with that of the control group (2.62±0.19). Magnesium (0.83±0.08) and phosphorus (1.13±0.17) showed a statistically non-significant ($P>0.05$) decrease in the petrol station attendants when compared with that of the control group (0.86±0.09 and 1.14±0.22) respectively.

Table 2 shows the results of the effect of duration of exposure to petrol fumes on mean values of serum calcium, magnesium and phosphorus levels of study subjects. The result revealed that calcium (2.13±0.13) and magnesium (0.77±0.65) showed statistically significant ($P<0.05$) decrease in the petrol station attendants exposed to petrol fumes for a period of ≥ 5years as compared with that of the control (unexposed) group (2.62±0.19 and 0.867±0.09) respectively. Phosphorus level showed a non-significant difference ($P>0.05$) when compared with that of the control group.

Table 3 shows the result of the comparison of the effect of petrol fumes on serum calcium, magnesium and phosphorus levels in male and female petrol station attendants. The result revealed that calcium (Ca^{2+}) and magnesium (Mg^{2+}) show a statistically significant ($P<0.05$) increase in female than male petrol station attendants, while phosphorus (PO^{2+}) level in male and female did not show statistically significant ($P>0.05$) different in petrol station attendants.

Table 1: Statistical results of the effect of petrol fumes on serum calcium, Magnesium and Phosphorus levels of study subjects.

Parameters	Control	Petrol Station Attendants	P-Value	Remarks
Calcium (mmol/L)	2.62±0.19	2.40±0.42	0.02	S
Magnesium (mmol/L)	0.86±0.09	0.83±0.08	0.217	NS
Phosphorus (mmol/L)	1.14±0.22	1.13±0.17	0.955	NS

Results are expressed as Mean ± Standard Deviation. NS= Non-significant; S= Significant.

Table 2: Statistical results of the Effect of Duration of Exposure on Serum Calcium, Magnesium and Phosphorus levels of study subjects.

Parameters Station	Control	Petrol Station Attendants < 2years	Petrol Station Attendants 2-5years	Petrol Attendants ≥5years
Calcium (mmol/L)	2.62±0.19	2.63±0.25	2.57±0.44	2.13±0.13*
Magnesium (mmol/L)	0.86±0.09	0.89±0.03	0.80±0.05	0.77±0.65*
Phosphorus (mmol/L)	1.148±0.22	1.24±0.08	1.10±0.08	1.11±0.07

Values with (*) superscript are significantly different from the control value at $p< 0.05$.
Results are expressed as Mean ± Standard Deviation.

Table 3: Comparison of the Effect of Petrol Fumes on Serum Calcium, Magnesium and Phosphorus Levels in Male and Female Petrol Station Attendants.

Parameters	Male Petrol Station Attendants	Female Petrol Station Attendants	P-value
Calcium (mmol/L)	2.10±0.44	2.38±0.39	0.031
Magnesium (mmol/L)	0.78±0.04	0.92±0.08	0.043
Phosphorus (mmol/L)	1.06±0.11	1.10±0.20	0.766

Results are expressed as Mean ± Standard Deviation. $p< 0.05$ is considered significant.

DISCUSSION

Increased exposure to harmful chemicals like benzene, toluene, xylene, ethyl benzene, and trace amounts of heavy metals such as lead in petroleum portend serious health risks to human.[7] This study was carried out to ascertain the effect of petrol fumes on serum calcium, magnesium and phosphorus levels. The result obtained in this study have confirmed that changes occur in the serum concentration of some micronutrients among petrol attendants. The alterations were dependent on duration of exposure. Alteration in the tissue level or bioavailability of such micronutrients could play a significant role in health as well as in the pathology of some disorders like myocardial failure, respiratory failure, osteomalacia, rickets, osteoporosis and fractures.[16]

Calcium is very essential in muscle contraction, oocyte activation, building strong bones and teeth, blood clotting, nerve impulse, transmission, regulating heart beat and fluid balance within cells. Long term of calcium deficiency can lead to oestoporosis in which the bone deteriorates and there is an increased risk of fractures.[11] The primary factor in the regulation of extracellular calcium is parathyroid hormone (PTH). It acts on the skeleton, small intestine, and kidney and interrelates with vitamin D and calcitonin to maintain the extracellular calcium concentration within narrow limits. Parathyroid hormone increases serum calcium by increasing the activity and number of osteoclasts, inducing bone resorption, and consequently mobilizing calcium from bone; augmenting calcium reabsorption by the kidney; and by increasing calcium absorption by the small intestine, indirectly, due to greater production of l,25(OH)2 vitamin D3. The consequences of most disturbance of calcium can be predicated from the knowledge of actions of parathyroid hormones (PTH) on bone, intestinal cells and renal tubular cells. Results from this current study showed that the mean value of calcium (Ca^{2+}) was significantly ($P<0.02$) reduced statistically in the petrol station attendants when compared with that of the control group. This finding is in agreement with

Awodele *et al.*,[9] who reported a statistical derangement in calcium levels of gas station workers. This reduction in calcium could be attributed to the effect of the toxic chemicals present in petroleum on parathyroid hormone activity thereby altering renal and intestinal reabsorption of calcium, which in turn decreases the level of extracellular calcium.

This study also showed a statistically significant ($P<0.05$) reduction in the calcium (Ca^{2+}) and magnesium (Mg^{2+}) levels of petrol station attendants for ≥ 5 years when compared with that of the control group as shown in table 2. This reduction in calcium (Ca^{2+}) and magnesium (Mg^{2+}) could be attributed to the fact that a decreased calcium level which in most times associated with a decreased magnesium level which may presents clinical manifestations like bone hunger syndrome, rhabdomyolysis, weakness, fatigue, muscle cramps, tetany, numbness, seizures and arrhythmias.[20] This finding concurred with Johnson and Umoren.[21] who reported that prolonged exposure to harmful chemicals in the petrol fumes would result in more health complication. However, Phosphorus (PO^{2+}) showed a non-significant ($P>0.05$) difference when compared with that of the control group. This finding may be indicative that exposure to petrol fumes has no significant effect on phosphorus level. However, it has a considerable effect on bones and this effect becomes more significant with a prolonged exposure.

Furthermore, the study revealed a statistically significant ($P<0.05$) increase in the calcium (Ca^{2+}) and magnesium (Mg^{2+}) levels in female than male petrol station attendants, while phosphorus (PO^{2+}) level in male and female did not show statistically significant ($P>0.05$) different in petrol station attendants (Table 3). Our finding is in agreement with Johnson and Umoren[21] who reported that female petrol station attendants are more prone to health challenges associated with exposure to petrol fumes.

Imbalances of micronutrients such as calcium, magnesium and phosphorus mostly are self-corrected and have no ill effects. However, these imbalances can portend serious consequences on normal health if left unregulated. In this study, we observed that serum levels of Ca^{2+}, Mg^{2+}, and PO^{2+} were within the normal reference range of an apparently healthy individuals. Interestingly, it was observed that the workers, although had the measured analyte levels in the normal healthy ranges but had lower levels of Ca^{2+}, Mg^{2+}, and PO^{2+} as compared to the control groups.

CONCLUSION

This study revealed that inhalation of petrol fumes by petrol station attendants for a duration of ≥ 5 years alters the serum concentration of calcium, magnesium and phosphorus and may adversely affect the bone which becomes more significant with prolonged exposure.

CONFLICT OF INTERESTS

The authors did not declare any conflict of interest

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