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Biochemical indices of calcium homeostasis as a biomarker of mixed toxic occupational exposure.Adunmo, G.O.*¹, Anetor, J.I.², Adesokan, A.A.¹, Adunmo E.O.¹*Department of Medical Biochemistry, College of Health Sciences, University of Ilorin¹, Department of Chemical Pathology, College of Medicine, University of Ibadan², Department of Family Medicine, University of Ilorin Teaching Hospital, Ilorin³.**Author for Correspondence *: +234/godwinadunmo@yahoo.com***Abstract**

Exposure to a single chemical and associated disorder in occupational environment has received a tremendous attention but unlike laboratory animals, people hardly get exposed to a single chemical agent and thus the need to examine possible alteration in biochemical parameters in the subjects exposed to mixed chemicals. Alteration in calcium homeostasis has been associated with cell transformation in the process of carcinogenesis. The aim of this study was to explore the effect of occupational chemical exposure on calcium homeostasis. Sixty-six (66) exposed subjects (aged 21-70) were selected. They comprised mechanics (41), panel beaters (12), welders (7) and painters (6) within Ibadan metropolis working together within the same environment. The mean duration of occupational exposures was 14.85± 8.85 years. Twenty-five males, apparently healthy non-exposed individuals, were observed as controls. Biochemical indicators of calcium homeostasis were evaluated in all subjects. Total Calcium, albumin, total protein and uric acid were determined spectrophotometrically, while ionized calcium and globulin were calculated. Ionized calcium level was significantly decreased ($p < 0.05$) in the exposed group compared with the controls. Total calcium though reduced in the exposed group compared with controls was not significantly altered ($p > 0.05$). Inorganic phosphate, like ionized calcium was significantly decreased in the exposed groups compared with the controls ($p < 0.05$). Alkaline phosphate and globulin were significantly raised ($p < 0.05$) in the exposed group. Uric acid was not significantly altered

($p > 0.05$). The results support alteration in calcium homeostasis in the exposed group and might be associated with cell transformation and the risk of carcinogenic process.

Keywords: Albumin, alteration, Calcium, chemical, exposure, homeostasis

Introduction

Global concern has been raised in recent years over the adverse effects that may arise from exposure to chemicals in the environment or the workplace. These include heavy metals such as lead, cadmium, mercury, arsenic as well as pesticides, solvents and other organic toxicants that have been extensively studied (Ana-mania *et al*, 2005; Anetor *et al*, 2005).

Several factors that may complicate the toxicological evaluation of chemical mixtures as two or more chemicals can have additive effects on the same site or altering the same system by different mechanisms. These effects may be antagonistic or synergistic, while some may have no interaction but act independently. Those without interaction may result in exposed individuals experiencing the sum total of the different organ toxicities of different contaminants (David *et al* 1998; Pounds and Rosen, 1988). Some of the major broad categories of human diseases that are suspected to result from exposure to occupational chemical toxicants are cancer, immune system defects, reduced intelligence quotient, behavioral abnormalities, decreased fertility, altered sex hormone balance, altered metabolism and specific organ dysfunction (Lybarger *et*



al.,1993). Almost every organs/system may be affected by one or more substances commonly found in occupational environment but when

expressed in an individual are difficult to ascribe with certainty to a particular exposure.

Table1 : Occupation and chemicals workers are exposed to.

Occupation	Benzene	Gasoline	Minerals acid	Ethylene	Toluene	Phenyle mercury	Lead	Cadmium	
Auto Mechanics		+	+	+	-	+	-	+	+
Panel beating		+	+	-	+	-	+	+	+
Battery charging		-	-	+	-	-	-	+	+
Welding		+	-	-	+	-	-	+	-
Painting		+	+	-	+	+	+	+	+
Auto /electrical		-	-	-	-	-	-	+	-

Anetor *et al.*,2009.

The close structural and functional relationship between the regulation of ions, cell injury and cell death has been of considerable interest for many years (Bon venture, 1988; Trump and Brezeck, 1987). There is a robust evidence that the deregulation of calcium homeostasis in the cell might be a primary event in cell transformation and carcinogenic process. Calcium occupy a prominent position in signal transduction system of the cell by virtue of its participation in a wide range of physiological functions and biological events associated with genetic expression, cell proliferation, apoptosis, cell differentiation and morphogenesis. This alteration in calcium homeostasis could lead to profound deregulation signal transduction with wide range of effects on the life of the cell and its biological behaviors. Alteration in the signaling process arising from alteration in calcium homeostasis and metabolism have been suggested to be the primary events in cell transformation and (chemical) carcinogenesis (Sherbert, 2000). Due to the central role of calcium in diverse functions of tissues, organs and cells, calcium homeostasis and calcium mediated process may prove to be critical cellular and molecular targets for a diverse range of chemical toxicants. Majority of toxic effects can be linked to a single chemical toxicants but few have been related to mixed chemical

exposure in an occupational environment (Anetor *et al.*, 2009).

The purpose of this study was to investigate biochemical indicators of calcium homeostasis in occupational exposure to mixed toxicants.

Materials and methods

Study population

The study population comprised of 66 males mean age of 34 ± 10.34 (range 21-70) years. They comprised of various artisans exposed to gasoline fumes and metal pellets within Ibadan Metropolis. The group was made up of, Auto mechanics (41), Panel beaters (12), Welders (7) and Painters (6). They were classified into 3 groups according to duration of exposure. Twenty-five males mean age 32.15 ± 8.23 (range 24-59) years apparently healthy non-exposed individuals were monitored as controls.

Exclusion criteria

Exclusion of individuals was based on the following;

1. History of degenerative disease such as cancer, cardiovascular disease, rheumatoid arthritis /osteoarthritis
2. History of chronic diseases such as diabetes mellitus, renal and liver disease.
3. Alcoholics and smokers



Data collection

Data collection was by personal interview using a structured questionnaire containing some of the following entries for each subjects, age, gender and length of years in the occupation. A signed informed consent form was obtained from the subjects

Collection of blood samples

Ten milliliters of fasting venous blood were collected using standard procedures. The samples were analyzed using Hitachi 902 auto analyzer using Roche test kits (Roche Diagnostics, Germany) while potassium was determined by standard flame emission photometer.

Estimation of ionized calcium

Ionized calcium was computed by employing the method of McLean and Hastings (1935) as adopted by Beeler and Catron (1983). Using the following formular.

$$\text{Ionized Calcium (mg/d)} = \frac{(\text{Ca(mg/dl)} \times 6) - (\text{SPr(g/dl)} \times 0.33)}{\text{SPr (g/dl)} + 6}$$

Where Ca = Total Calcium

Where Pr = Total protein

Statistical analysis

The data were analyzed on a microcomputer using SPSS (statistical package for social sciences) software package. The summary descriptive statistics; mean (x) and standard

deviations (SD) of physical and biochemical parameters were computed. Pair t- test was used to investigate the significance of groups considered. All reported values in this study were expressed as means (X) + standard deviation. In all statistical analysis, a p- value of <0.05 was accepted as significant.

Result

The occupationally exposed group showed a significantly higher level of ALP concentration when compared with the control group ($p < 0.05$). Unlike ALP, the exposed group demonstrated a significantly lower level of inorganic phosphates when compared with the control group ($p < 0.05$). Similarly, there was a significantly lower level of ionized calcium in the exposed group when compared with the unexposed ($p < 0.05$). Table 2 shows the comparison mean level of indexes of calcium homeostasis and potassium between occupationally and the unexposed (controls) subjects. Potassium levels was not significantly different from the control groups ($p > 0.05$).

The result showed a significantly higher level of globulin in the exposed group compared with the control subjects ($p < 0.05$) but there was a non-significant ($p > 0.05$) changes in increase uric acid, albumin, and total protein levels in both groups. Table 3 shows a comparison of the level of uric acid, albumin, total protein and globulin between the occupationally exposed and the control subjects.

Table 2: Comparison mean level of indexes of calcium homeostasis and potassium between occupationally and the unexposed (controls) subjects.

Parameter	Exposed N = 66	Non exposed N = 25	t- value	p- value
Total calcium (mg/dl)	9.42 ± 0.71	9.64 ± 0.86	1.14	>0.05
Ionized calcium (mg/dl)	4.09 ± 0.42	4.32 ± 0.48	2.16	<0.03
ALP (U/L)	79.44 ± 45.29	57.44 ± 13.84	2.47	<0.02
Inorganic phosphate (mg/dl)	3.84 ± 0.76	4.24 ± 0.88	2.25	<0.03
Potassium (mmol)	3.73 ± 0.74	3.84 ± 0.67	0.66	>0.05

Table 3: Comparison of the level of uric acid, albumin, total protein and globulin between the occupationally exposed and the control subjects

Parameters	Exposed N = 66	Non exposed N = 25	t- value	p- value
Uric acid (mg/dl)	5.51 ± 1.12	5.25 ± 0.61	1.0032	>0.05
Albumin (g/d)	4.10 ± 0.52	4.20 ± 0.37	0.96	>0.05
Total protein (g/dl) ¹	7.17 ± 0.78	6.92 ± 0.89	1.30	>0.05
Globulin (g/dl)	3.06 ± 0.69	2.71 ± 0.68	2.22	<0.05



Discussion

The data from this study suggests an alteration in the biologically active fraction of calcium, ionized calcium. Although the total calcium was lowered in the exposed subjects compared with control. The difference however was not statistically significant ($p > 0.05$). The reduced ionised calcium which is the biologically active fraction indicates an alteration in the metabolically active fraction of calcium homeostasis. Our finding is consistent with the findings of Rosey and Riggney (1977) and Anetor *et al.* (2005) which indicated a decreased level in ionized calcium in a population exposed to lead, a common occupational chemical. They suggested that chemical toxicants such as lead is a biochemical analogue of calcium interfere with calcium in several metabolic pathways (Goyer, 1995). Several mechanisms have been proposed to explain this observation but most accepted is their interference with the final metabolism of vitamin D to the active metabolites, calcitriol (1,25 dihydroxyl cholecalciferol) a hormone required for the adequate uptake of calcium metabolism. Calcitriol is an hormone required for the adequate uptake of calcium from the duodenum and phosphate from the jejunum. Thus, an alteration in the metabolism of vitamin D will impair the calcium absorption and simultaneous significant reduction in inorganic phosphate (Goyer, 1993; Tiez, 2012). Inorganic phosphate is an intracellular anion whose level in the tissue may be altered during cell membrane damage. In this study, a reduction in phosphate level in the exposed group was observed. The significant reduction in phosphate level observed in this study may suggest that parathyroid hormone (PTH) is involved in the calcium phosphate homeostasis in this study. Decreased ionized calcium level through a feedback mechanism, increases the secretions of parathyroid hormones (PTH) by the parathyroid gland. PTH exerts its action by interacting with PTH receptors located in the plasma of target cells such as bone and kidney. PTH increases calcium reabsorption in the distal tubule of nephron and decreased the reabsorption of the phosphates at the proximal tubules, resulting in an increased excretion of phosphate in the urine (phosphaturia) (Mundy and Guise, 1999).

The raised ALP level may suggest an adaptive response to the low phosphate level. This results in the breakdown of organic phosphate to raise the concentration of inorganic phosphate which may culminate in ultimately alteration in signal transduction and thus altering the cell cycle with its attendant pathologies. This increase in ALP may arise as a result of an attempt at osteogenesis, which in the phase of decreased ionized calcium and inorganic phosphate is inappropriate, leading to increased release of osteoblasts and in turn ALP. The observed raise uric acid in this report may have arising from action of the enzyme Xanthine oxidase on xanthine and hypoxanthine. These purines (adenine and guanine) are present in RNA and DNA. An increase in the uric acid though not significant may indicate indirect effect of toxicant on the metabolism of both RNA and DNA. It has been reported (Ercal *et al.*, 2000; Pounds, 1990) that chemical toxicants induces cellular oxidative stress thus causing an increased production of reactive oxygen species (ROS) such as hydroxyl radicals ($\text{OH}\cdot$), superoxide radicals ($\text{O}_2\cdot^-$) or hydrogen peroxide (H_2O_2). Enhanced generation of reactive oxygen species can overwhelm the cell's intrinsic antioxidants defences resulting in oxidative stress. Cells under oxidative stress display various dysfunction due to lesions caused by ROS to lipids, protein and DNA. Thus the raised level of uric acid, though not significantly, may be in part an antioxidants response to these generated reactive oxygen species. Simic and Jovanic (1989) have also associated this form of increase with DNA repair due to DNA damage by reactive oxygen species.

Conclusion

There is increased burden of exposure to chemicals at the work place, especially in developing countries, where precautionary measures against direct contacts with these chemicals are not taken into consideration which can lead to chemically induced diseases. In this study, alteration in indices of calcium homeostasis is observed. This has many implications in the pathogenesis of many uncommunicable diseases especially cancer and osteoporosis.



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