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Association of Vitamin D and Calcium Levels in E-waste Workers and Environmentally Exposed Participants in Southwestern Nigeria

***IGHARO, OG; IDOMEH, FA**

Department of Medical Laboratory Sciences, School of Basic Medical Sciences, College of Medical Sciences, University of Benin, Benin City, Nigeria.

***Corresponding Author** address: osaretin.igharo@uniben.edu; Tel: +2348038664896*

ABSTRACT: Exposure to e-waste either by occupation or through the environment have been implicated in cancer development. The growing influx of e-waste to Nigeria and inadequate recycling, part salvaging, distribution and disposal of discarded or obsolete electronic devices is creating new sets of environmental and public health challenges. This study investigated the effect of exposure on vitamin D (25(OH) Vit D), total and ionized calcium levels in workers and individuals environmentally exposed to e-waste in Lagos, Benin and Ibadan cities in Southwestern Nigeria. Six hundred and thirty-two participants from three major cities in Nigeria were recruited for this study. They included e-waste workers, environmental e-waste exposed individuals and age matched unexposed individuals. 25(OH) Vit D was determined using ELISA, while total and ionized calcium by colorimetric method. Total calcium (tCa) in e-waste workers was significantly different from environmental exposed groups ($p=0.000$) while ionized calcium (iCa) was significantly increased for both e-waste exposed groups when compared with the unexposed group. 25(OH)Vit D level was significantly increased in e-waste workers compared with environmental and unexposed populations ($p<0.001$). Optimal levels of tCa, iCa and vitamin D in the e-waste exposed population may offer some forms of defense against the severity of existing and undiagnosed cancers or reduce the risk of cancer development in the e-waste exposed participants. However, e-waste may unfavourably modulate these expected beneficial roles in the e-waste exposed populations with continued exposure.

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E-waste is a rapidly growing problem of the world and an estimated 20-50 million tons is generated per year of which 75-80% is shipped to Asia and Africa (with Nigeria been one of such countries) for recycling and disposal (Adaramodu *et al*., 2012). High incidence of cancer has been associated with some forms of occupational exposure to carcinogens. The majority of cancers, some 90–95% of cases, are due to environmental factors, the remaining 5–10% are due to inherited genetics (Parkin *et al*., 2011). Common environmental factors that contribute to cancer death include tobacco (25–30%), diet and obesity (30–35%), infections (15–20%), radiation (both ionizing and non-ionizing, up to 10%), stress, lack of physical activity and environmental pollutants, e-waste being an example (Parkin *et al*., 2011). A low vitamin D status and inadequate calcium (Ca) intake are important risk factors for various types of cancers (Park *et al*., 2009). Ca is a very important macro element and poor Ca nutrition is a significant risk factor for total cancer incidence (Park *et al*., 2009). The importance of Ca in carcinogenesis derives from its participation in regulating cell proliferation, differentiation, and apoptosis (Mathiasen *et al*., 2002). Evidence is available that Ca at least partially exerts its anti-carcinogenic effects through vitamin D. For example, Ca is one of the key mediators of apoptosis induced by vitamin D compounds in breast cancer cells (Mathiasen *et al*., 2002). Vitamin D has been inversely associated with incidence of many chronic diseases (20) and its insufficiency plays important pathogenic role in many malignancies (Garland *et al*., 2006). 25(OH) 2D, the biologically active form of vitamin D, exerts its effects mainly through binding to nuclear vitamin D receptor (VDR) and further binding to specific DNA sequences, namely vitamin D response elements. Through this genomic pathway, 1, 25(OH) 2D modulates expression of specific genes in a tissue-specific manner (Wels, 2004). Studies have shown that 1, 25(OH) 2D can inhibit cellular proliferation, induce differentiation and apoptosis, and inhibit angiogenesis in normal and malignant breast cells (Grau *et al*., 2003). Owing to the growing influx of e-waste to Nigeria and an estimated 75% of the 400,000 computer monitors or 175,000 TV sets entering Lagos, Nigeria each month alone, and the accompanied

**Corresponding Author address: osaretin.igharo@uniben.edu; Tel: +2348038664896*

inadequate recycling, part salvaging, poor distribution and disposal of discarded or obsolete electronic devices creates new set of environmental and public health challenges in Nigeria (Terada, 2012). This study was aimed at investigating the effect of e-waste exposure on 25(OH) Vit D, total and ionized calcium levels in workers and individuals environmentally exposed to e-waste in Lagos, Benin and Ibadan cities in Southwestern Nigeria.

MATERIALS AND METHOD

Study Design: This was a cross-sectional study with purposive approach in the recruitment of participants into the study groups. The groups comprised of e-waste occupationally exposed participants (e-waste workers), participants trading or working around the e-waste repair/ recycling sites/locations considered for the study (environmentally exposed), and non-occupationally exposed, apparently healthy participants (unexposed or control participants).

Study Areas: The study was carried out in three urban cities (Lagos, Ibadan and Benin). These have been identified and reported as high impact locations for e-waste activities in South–West Nigeria (Osibanjo and Nnorom 2007).

Study Participants: A total of six hundred and thirty two participants were enrolled into the study: viz, three hundred and eighty-one e-waste workers; one hundred and twenty environmental e-waste exposed participants and one hundred and thirty-one age-matched unexposed individuals, serving as controls.

E-waste workers: Male waste electric and electronic equipment (WEEE) workers who were involved daily with four main tasks, purchasing/reception, dismantling, repair and resale, formed the group of e-waste workers enrolled into the study.

Environmentally Exposed Participants: The environmentally exposed group comprised of traders and non-e-waste workers involved in work and business activities around the e-waste high impact areas within the study location.

Unexposed Participants: Non-occupationally and minimally environmentally exposed, and apparently healthy age- and sex -matched participants formed the unexposed or control group.

Sample Collection and Preservation: Prior to blood collection, participants were urged to abstain from using herbal medications, drugs and vitamin/mineral supplements for 12 - 24hours. About ten (10) millilitres (mL) of venous blood was collected from each participant using standard phlebotomy technique. Blood sample obtained was dispensed into ethylene diamine tetra acetic acid (EDTA) anticoagulant specimen bottles (5 mL). Another 5 millilitres was dispensed into anticoagulant-free specimen bottles to obtain serum. Blood samples were allowed to clot, centrifuged at 3000 revolution per minute for 3 minutes and serum collected and stored in another anticoagulant-free bottle. Samples were immediately analyzed and when delay anticipated, serum samples were kept frozen (0 to -4^{o}C) until analysis.

Determination of Total and Ionized Calcium, and 25(OH) Vitamin D: **Quantification** of 25 (OH) Vitamin D was done using enzyme linked immunosorbent assay (ELISA) as described by Holick. (Holicks, 2000). While total calcium was estimated according to Ray-Sarkar and Chauhan (1967).

Determination of ionized calcium: Ionized calcium was calculated (in mg/dL) with the formula:

$$iCa = [0.9 + (0.55 \times tCa - 0.3 \times albumin)]$$

$$iCa = (6 \times tCa - TP/3)/(6 + TP).$$

Where tCa is in mg/dL, albumin and TP are in g/dL. (Toffaletti and Wildermann, 2001).

Statistical Analysis: Analysis was carried out using SPSS version 21 IBM.

RESULTS AND DISCUSSION

Total and ionized calcium and 25 (OH) vitamin D levels in e-waste exposed and unexposed participants in the different study locations: The level of total calcium (tCa) in e-waste workers was increased but not significantly different from levels in unexposed group (Table 1), but both were significantly increased when compared with environmentally exposed group ($p=0.000$). Ionized calcium (iCa) was significantly increased in e-waste workers when compared with the unexposed population and environmentally exposed when compared with unexposed population ($p=0.002$). Notably, 25(OH) vitamin D level was significantly increased in e-waste workers compared with the environmentally exposed and unexposed populations ($P<0.001$). Table 2 reveals that tCa and iCa in Lagos e-waste workers significantly increased when compared with Benin e-waste workers. However, tCa did not vary significantly between Ibadan and Lagos e-waste workers, also iCa did not vary in Benin and Ibadan e-waste workers. Levels of 25(OH) Vitamin D incrementally varied significantly

from Ibadan to Benin and to Lagos e-waste workers, (p=0.039). In the environmentally exposed group, Lagos and Ibadan e-waste workers had significantly increased iCa than their Benin counterparts, (p=0.001). The level of tCa was significantly increased in the Ibadan group compared with the Benin and Lagos environmental exposed participants. In addition, vitamin D was significantly higher in Benin environmental exposed participants than the Lagos and Ibadan groups. In the unexposed group, tCa, iCa, and vitamin D were significantly increased in Ibadan group compared with other locations.

Table 1. Total and Ionized Calcium, and 25(OH) vitamin D levels in e-waste exposed and unexposed participants

All Participants					
Parameters	**E-waste Workers (n=381)**	**Environmentally Exposed (n=120)**	**Unexposed (n=131)**	***F* value**	***P* value**
tCalcium (mmol/L)	2.81^{A}±0.06	2.44^{B}±0.08	2.64^{A}±0.05	8.70	0.000*
iCalcium (mmol/L)	1.40^{A}±0.03	1.39^{A}±0.03	1.27^{B}±0.03	6.56	0.002*
25(OH) Vitamin D, (nmol/L)	164.75^{A}±8.69	125.70^{B}±5.44	118.14^{B}±6.29	12.95	0.000*

Table 2. Total and Ionized Calcium, and 25(OH) vitamin D levels in e-waste workers, environmentally exposed and unexposed participants in the different study locations

	E-waste Workers			***F* value**	***P* value**
Parameters	**Benin-MiIA (n=83)**	**Ibadan-MoIA (n=120)**	**Lagos-HiIA (n=178)**		
tCalcium, (mmol/L)	2.58^{B} ±0.05	2.84^{A} ±0.13	2.98^{A}±0.12	18.14	0.000
iCalcium, (mmol/L)	1.39^{B} ±0.03	1.32^{B} ±0.03	1.51^{A} ±0.10	18.14	0.000
25(OH) Vitamin D, nmol/L)	155.54^{B} ±2.64	94.89^{C} ±8.36	215.67^{A} ±19.27	3.32	0.039
Environmentally Exposed					
Parameters	**(n=30)**	**(n=38)**	**(n=52)**	***F* value**	***P* value**
tCalcium (mmol/L)	2.36^{B} ±0.06	3.08^{A} ±0.21	2.53^{B} ±0.08	9.11	0.000
iCalcium, (mmol/L)	1.27^{B} ±0.02	1.50^{A} ±0.07	1.42^{A} ±0.04	7.31	0.001
25(OH) Vitamin D (nmol/L)	171.02^{A} ±2.84	61.63^{B} ±4.17	141.45^{B} ±9.08	132.87	0.000
Unexposed (Controls)					
Parameters	**(n=42)**	**(n=50)**	**(n=40)**	***F* value**	***P* value**
tCalcium (mmol/L)	2.44^{B} ±0.05	2.78^{A} ±0.14	2.23^{B} ±0.08	4.92	0.000
iCalcium, (mmol/L)	1.27^{B} ±0.03	1.48^{A} ±0.09	1.14^{C} ±0.02	14.33	0.000
25(OH) Vitamin D (nmol/L)	122.86^{A} ±4.62	141.79^{A} ±24.41	97.36^{B} ±5.11	3.82	0.024

MiIA = Mild e-waste impact area; MoIA = Moderate e-waste impact area; HiIA = High e-waste impact area

Vitamin D and calcium (Ca) are metabolically interrelated and highly correlated dietary factors. Experimental studies have shown their anti-carcinogenic effects due to their participation in regulating cell proliferation, differentiation, and apoptosis in normal and malignant cells (Mathias and Michael, 2013). In recent years, the nutritional risk factors in special consideration to vit D have gained much importance. A growing body of evidence suggests the protective mechanism of vit D against breast cancer by autocrine/paracrine manner (Afrozul and Nighat, 2017). Calcitriol, the hormonally active form of vit D exerts multiple anti-proliferative, pro-apoptotic, and pro-differentiating actions on various malignant cells and retards tumor growth in animal models of cancer (Afrozul and Nighat, 2017). Laboratory studies have demonstrated that vit D3 and its analogs inhibit cell proliferation and promote apoptosis in cancer cells in culture (Afrozul and Nighat, 2017). Lagos e-waste workers were exposed to more varieties of e-waste when compared with e-waste workers of other local and a corresponding lowered vit D level, which, could be indicative of preclinical and clinical findings which hypotheses that low levels of vit D are linked to an increased risk of breast cancer (BC) (Afrozul and Nighat, 2017). Thus, suggesting that occupationally exposed e-waste workers in Lagos may readily dispose to cancer than their counterparts in Benin and Ibadan over time.

Anti-carcinogenic effects of vit D are mediated via the estrogen pathway by down regulation of the estrogen receptor (ER), which inhibits cancer cell proliferation, induces cell apoptosis, and prevents carcinogenesis in vitro and in animal models. Data from both epidemiological and experimental studies suggest an association of vit D deficiency with BC prevention and survival. There exists mounting evidence that individuals with higher 25(OH)D concentration at the time of cancer diagnosis have better cancer-specific and overall survival rates, suggesting that cancer-affected people should raise their 25(OH)D concentrations (Afrozul and Nighat, 2017). This study shows increased levels of vit D in e-waste exposed groups, suggestive of vit D anti-carcinogenic property and is in line with the study of Rasha and colleagues (Rasha *et al*., 2017) who showed vit D deficiency to be more common in patients with BC when compared to age matched control groups,

thus, indicating a strong association between breast cancer risk and serum levels of vit D.

The study of Imtiaz *et al* (2012) reported vit D deficiency in 95.6% of Indian patients with BC and 77% in control group is also supportive of this study. Although 1,25-dihydroxyvitamin D is the active form, it is widely accepted that the measurement of circulating 25(OH) D provides better information in patients vit D status (Slatter *et al*., 2001). Low levels of circulating 25(OH) vit D have been hypothesised to decrease the local production of $1,25(OH)_2D$ within the breast tissue increasing the risk of BC development (Rasha *et al*., 2017). Additionally, vit D has been linked to promoting cellular differentiation, decreasing tumor cell growth, stimulating apoptosis, and reducing angiogenesis (Thorne and Campbell, 2008). Ca has been hypothesised to play a dual role in proliferation/activation and apoptosis of cancer cells (37). Cytosolic Ca^{2+} control is by three main general categories. These mechanism by controlling cytosolic Ca^{2+} regulate proliferation, activation and apoptosis. Generally, small elevations of cytosolic Ca^{2+} have being hypothesised to increase cell proliferation whereas sustained elevations may induce apoptosis (Schwarz *et al*., 2013). It is possible that Ca intake has impact on cancer risk with some observational studies suggesting that a high intake of Ca and/or vit D are associated with reduced risk of colorectal and breast cancer (Sarah *et al*., 2013). Lappe and colleagues reported Ca monotherapy to causing 47% decrease in total cancer risk (Lappe *et a*l., 2007). In contrast Bolland and colleagues found no effect of Ca monotherapy on total cancer incidence (Bolland and Reid, 2008), Avenell et al. in a randomised evaluation reported no effect of Ca on mortality (Avenell *et al*., 2012) and Chlebowski and colleagues also reported no effect of Ca plus vit D on the risk of colorectal or breast cancer (38). Although recent reanalysis of the study of Chlebowski and colleagues report significant interaction between treatment allocation, personal or vit D supplement and the risk of total, breast and colorectal cancers (Sarah *et al*., 2013). We report increased Ca^{2+} levels in exposed groups. This increase combined with vit D levels may have accounted for the apparently non-carcinogenic state of participants. In conclusion, the optimal levels of tCa, iCa and Vit D in the e-waste exposed population may offer some forms of defense against the severity of existing and undiagnosed cancers or reduce the risk of cancer development in the e-waste exposed participants. The protective role of vit D as previously highlighted in combination with Ca may account for the defense against existing and undiagnosed cancer. However, the antagonistic effects of toxic metals from e-waste on calcium ions and vitamin D may unfavourably modulate these expected beneficial roles in e-waste exposed populations with continued exposure.

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