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Endocrine Disruptors-Arsenic, cadmium and lead in pre and postmenopausal black women with breast cancer.

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Abstract
Background: The involvement of toxic metals in adiposity has been suggested to be contributory to the high incidence of breast cancer, particularly in sub-Saharan Africa. This study is aimed at evaluating serum arsenic, cadmium and lead in relation to adiposity and blood pressure in Nigerian women with breast cancer.
*Methodology***:** The study comprised 85 women newly diagnosed with breast cancer pre-therapy (cases) matched with 84 apparently healthy women without breast cancer (controls) according to age and menstrual phase. Arsenic (As), cadmium (Cd) and Lead (Pb) levels were determined by atomic absorption spectrophotometry. Blood pressure and anthropometry were determined by standard methods. Data analysed by Student's t-test and Pearson correlation coefficient were considered statistically significant at $p<0.05$.
Results: Cd and Pb levels were significantly higher in cases, compared with controls ($p<0.05$). Waist circumference (WC), hip circumference (HC), weight, height, waist hip ratio (WHR), waist height ratio (WHtR) were significantly higher in cases compared with controls ($p<0.05$). Cadmium positively correlated with diastolic blood pressure while FT_4 inversely correlated with arsenic in the cases ($p<0.05$).
Conclusion: Observations in this study suggest the involvement of these toxic metals in adiposity which could be involved in breast carcinogenesis.

Keywords: *Lead, cadmium, arsenic, breast cancer, blood pressure, adiposity.*

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Introduction

Breast cancer is the most frequent cancer among women and also the leading cause of cancer mortality in women globally. An estimated 1.5 million new cases of breast cancer worldwide was reported in 2002 [1]. In Nigeria, an increase in incidence of breast cancer has been reported [2]. Recent findings have suggested the contribution of environmental factors to the high incidence of breast cancer [3]. This is due to the increased use of various metals in industry, agriculture and medicine brought about by the current industrial revolution which has invariably led to wide spread environmental pollution [4].

Toxic metals-arsenic (As), cadmium (Cd) and lead (Pb) have been reported to adversely affect the endocrine signalling system and are referred to as endocrine disruptors (EDs). Endocrine disruptors are almost impossible to eliminate in the environment,

because they do not decompose, hence, they are absorbed into the human system through different routes. Arsenic and cadmium compounds are lipophilic, hence, they readily penetrate cell membranes [5]. On the other hand, cadmium can bind to protein to form a complex (cadmium-metallothionein) which is actively taken into the cell by endocytosis [6]. Lead may be absorbed by passive diffusion [7].

These toxic metals are a major source of oxidative stress, which is involved in the development of breast cancer [8]. Emerging reports indicate that these toxicants could influence adiposity, however, the exact mechanisms involved is not clear. Visceral adiposity has been strongly associated with an adverse metabolic risk including insulin resistance and a strong aetio-pathogenic factor for the development of type 2 diabetes mellitus [9]. These systemic effects could be involved in cancer biology [10]. Arsenic is a metalloid that is ubiquitous in the environment. Human exposure includes ingestion of contaminated food and water, inhalation of contaminated air and by dermal contact. Arsenic compounds are lipid soluble and within 24 hours of absorption are distributed throughout the body where they can bind to sulfhydryl (-SH) groups on proteins. Arsenic may also replace phosphorus in bone tissue and be stored for years [11]. Methylation efficiency in humans appears to decrease at high arsenic dose. Studies show that aging is associated with a diminishing capacity to methylate inorganic arsenic, resulting in increased retention of As in soft tissues [12] including breast tissues. Chronic exposure to As compounds has been associated with several types of cancer [13]. Arsenic interferes with oestrogen receptor, its non-cytotoxic concentrations significantly inhibited oestradiol receptor-regulated effects in human breast cancer MCF-7 cells [14].

Cadmium ranks close to lead as a metal of current toxicological concern [15]. It occurs in nature in association with zinc and lead. Extraction and processing of these metals often lead to environmental contamination with Cd. Although, smoking is a well-established source of cadmium exposure, the major route of cadmium exposure is ingestion of shellfish and certain food, particularly root vegetables, potatoes and grains (rice and wheat) grown on cadmium-rich soils. [16]. Cadmium is a known cumulative toxicant with a biological half-life of more than 10 years in humans [17]. Cadmium accumulation occurs in the adipose tissue, liver and kidneys [18]. Only a small fraction of inhaled or ingested Cd is excreted, resulting in increased body burden over time [19]. Chronic low Cd exposure will eventually result in accumulation to toxic levels [18]. Women tend to have higher Cd levels than men presumably because of lower iron stores, which increase Cd absorption. Thus, comparable environmental exposures to Cd may disproportionately affect women compared to men [20]. Significantly higher Cd levels were observed in breast tissue and biological media from women with breast cancer compared with controls, suggesting that exposure to Cd could be interpreted as a potential risk factor for breast cancer [21].

Lead has been reported as a metal that can be found in drinking water, which is of great public health concern [15]. Lead contamination in the environment, resulting in toxicity in several body organs and systems has been documented [22]. This is in spite of the fact that Pb in gasoline, food cans and in paints was banned in the United States between 1980 and 1990. Recent reports showed that enamel paints with very high levels of Pb were sold freely in Nigeria [23]. The association of Pb with breast cancer is inconsistent [24].

There is paucity of information regarding the association of As, Cd and Pb with adiposity in breast carcinogenesis in sub-Saharan Africa. This study was therefore designed to determine the serum levels of As, Cd and Pb in relation to adiposity in Nigerian women with breast cancer who have not started treatment.

Materials and methods

Study design

This study was a case-control study conducted in the Surgical Oncology Clinic of the Department of Surgery, University College Hospital, Ibadan. The study protocol was approved by the University of Ibadan and University College Hospital Health Review Committee (UI/EC/10/0193). Informed consent was obtained from the participants before their recruitment. Semi-structured pre-test questionnaire was administered to each participant to obtain data on demography, social, diet and reproductive history.

Study participants

One hundred and sixty-nine women aged 28-80 years were consecutively recruited for this study. Eighty-five were histologically confirmed breast cancer patients who had not commenced treatment (Cases). They were recruited from the Surgical Oncology Clinic of the Department of Surgery, University College Hospital, Ibadan, by a Consultant Surgical Oncologist. Eighty-four non-pregnant, apparently healthy women aged 28-80 years were recruited as controls. The controls were recruited at three Primary

Health Clinics (PHC) in Ibadan North Local Government Area of Oyo state (PHC, Idi Odundun, Agodi, PHC, Agbowo and Elderly Women/Widows Clinic, Agodi-gate). Their breasts were examined by trained nurses for the presence of any breast lump. They were asked if they felt any pain or had any discomfort in their breasts. Those that complained of pain, discomfort and/or had lump in their breasts were excluded from the study. Each of the cases was matched for age and menstrual phases (follicular, luteal and menopausal status) with the controls. Participants were reported as postmenopausal if they had stopped menstruating over the last twelve months [25]. Participants that had bilateral oophorectomy were also considered postmenopausal.

Inclusion criteria

Non pregnant, non- hypertensive participants with histologically confirmed breast cancer who had not commenced treatment and gave informed consent.

Exclusion criteria

Pregnant women and those who reported being on hormonal drugs (i.e. contraceptives), had other types of cancers and/or chronic diseases were excluded from the study. Postmenopausal women on hormone replacement therapy were also excluded.

Anthropometric indices

Anthropometric indices: weight, height, body mass index, waist circumference, hip circumference, waist-hip ratio, waist-height ratio were measured by standard methods described elsewhere [26].

Blood pressure (BP) measurement

Blood pressure was determined using a mercury sphygmomanometer and recorded to the nearest mmHg [28]

Sample collection

10mL of venous blood was aseptically obtained by venepuncture from participants and dispensed into plain bottle in the controls, and after diagnosis and histological confirmation of invasive ductal carcinoma pre-therapy in the cases. All samples were centrifuged at 500g for 5 minutes, the serum obtained was aspirated into clean vials and stored at -20^{o}C until analyses were done.

Toxic metals estimation

Serum arsenic, cadmium and lead were determined by atomic absorption spectrophotometry **(**Buck Scientific, 210 VGP. Atomic Absorption Spectrophotometer. Connecticut, USA).

Serum oestradiol, progesterone, FT_4 and TSH were determined by enzyme immune assay on TOSOH AIA System Analyzers (Tosoh Corporation, Tokyo 105-8623, Japan). Values for hormones; oestradiol, progesterone, FT_4 and TSH have been previously reported [27]. The reference intervals of hormonal assays are Oestradiol (E_2): Follicular Phase: 90-1100 (pmol/L), Luteal Phase: 90-1200 (pmol/L), Postmenopausal: d”170 (pmol/L). Progesterone: Follicular: d”2.8 (nmol/L), Luteal: 15-80 (nmol/L), Postmenopausal: d” 1.59 (nmol/L). Free Thyroxine (FT_4):10.6-21.0 (pmol/L). Thyroid Stimulating Hormone (TSH):0.38-4.31 (mIU/L). The limits of detection were 0.318 nmol/L for progesterone, 52.85 pmol/L for oestradiol, 1.29 pmol/L for FT_4 and 0.01 mIU/L for TSH. The intra-batch coefficients of variation were 11.3% for progesterone, 4.1% for oestradiol, 5.3% for FT_4 and 5.0% for TSH.

Statistical analysis

Data were analysed using Statistical Package for Social Scientists (SPSS) software, version 18. Student’s t-test was used for comparison of quantitative variables. Pearson correlation coefficient was used to find relationships between the quantitative variables. Two-tailed independent t-test of significance at 95% confidence limit with $p<0.05$, was considered significant.

Results

Table 1 shows age, anthropometric indices, blood pressure and toxic metals in premenopausal women with breast cancer (pre cases) and premenopausal women without breast cancer (pre controls). Waist circumference, hip circumference, weight, height, waist hip ratio, waist height ratio, systolic blood pressure, lead, cadmium and arsenic were significantly higher in premenopausal women with breast cancer compared with the controls ($p<0.05$).

. Table 2 shows age, anthropometric indices, blood pressure and toxic metals in postmenopausal women with breast cancer (post cases) and postmenopausal women without breast cancer (post controls). Weight, height, lead, cadmium and arsenic were significantly higher in postmenopausal women with breast cancer ($p<0.05$)

Table 3 shows age, anthropometric indices, blood pressure and toxic metals in women with breast cancer (cases) and women without breast cancer (controls). Waist circumference, HC, weight, height, WHR and WHtR, systolic blood pressure, lead, cadmium and arsenic were significantly higher in women with breast cancer ($p<0.05$).

Table 1: Comparison of Age, blood pressure and toxic metals in premenopausal women with breast cancer and controls.

Variable	Cases (n=54)	Control (n=53)	t	*P*
Age (years)	40.91±0.7	40.74±0.6	0.19	0.852
Toxic Metals				
Lead (µg/dL)	5.4±0.2	1.8±0.1	18.35	<0.001*
Cadmium (µg/dL)	0.04±0.00	0.01±0.00	18.79	<0.001*
Arsenic (µg/dL)	0.30±0.01	0.04±0.00	17.59	<0.001*
Hormones				
Oestradiol (pmol/L)	452.8±43.3	430.8±46.5	0.35	0.729
Progesterone (nmol/L)	12.3±2.6	8.8±2.2	1.02	0.309
FT_4 (pmol/L)	17.8±0.6	14.9±0.3	4.51	0.000*
TSH (mIU/L)	1.8±0.2	1.5±0.1	1.36	0.178
Anthropometric Indices				
Waist Circumference (cm)	88.5±1.4	78.3±1.3	5.32	<0.001*
Hip Circumference (cm)	100.5±1.5	95.9±1.0	2.51	0.014*
Weight (Kg)	68.0±1.9	60.1±1.3	3.44	0.001*
Height (m)	1.63±0.0	1.57±0.0	4.35	<0.001*
Body mass index (Kg/m^2)	25.7±0.7	24.5±0.5	1.40	0.164
Waist hip ratio	0.88±0.0	0.81±0.0	6.07	<0.001*
Waist height ratio	54.6±1.0	49.9±0.9	3.52	0.001*
Blood pressure				
SBP(mmHg)	122.96±1.4	119.04±1.2	2.06	0.042*
DBP (mmHg)	82.4±1.1	80.9±1.0	0.97	0.336

*Values are mean±SEM (Standard error of mean), n=number of subjects, t=Student's t-test, p=significance level, *=significant at p<0.05, SBP=Systolic blood pressure, DBP=Diastolic blood pressure, µg/dL=micrograms per decilitre, nmol/L=nanomole per litre, pmol/L=picomole per litre, mmHg=millimetre mercury*

Table 2: Comparison of age, blood pressure and toxic metals in postmenopausal women with breast cancer and controls.

Variable	Cases (n=31)	Control (n=31)	t	*P*
Age (years)	61.23±1.5	61.65±1.5	-0.19	0.843
Toxic metals				
Lead (µg/dL)	5.76±0.24	1.75±0.06	15.98	<0.001*
Cadmium (µg/dL)	0.05±0.00	0.01±0.00	15.99	<0.001*
Arsenic (µg/dL)	0.31±0.02	0.04±0.00	15.22	<0.001*
Hormones				
Oestradiol (pmol/L)	156.5±12.4	90.4±3.6	5.04	0.000*
Progesterone (nmol/L)	2.1±0.4	1.0±0.1	2.92	0.005*
FT_4 (pmol/L)	17.7±0.6	14.3±0.4	4.79	0.000*
TSH (mIU/L)	1.6±0.2	1.3±0.1	1.36	0.181
Anthropometric indices				
Waist circumference (cm)	92.2±1.7	89.8±1.5	0.97	0.337
Hip circumference (cm)	103.9±1.7	102.7±1.7	0.50	0.619
Weight (Kg)	71.4±2.2	65.6±1.7	2.10	0.010*
Height (m)	1.63±0.0	1.59±0.0	2.34	0.023*
Body mass index (Kg/m^2)	26.8±0.7	25.7±0.7	1.25	0.217
Waist hip ratio	0.89±0.0	0.88±0.0	0.09	0.480
Waist height ratio	56.6±1.2	56.5±0.9	0.09	0.930
Blood pressure				
Systolic BP (mmHg)	122.26±1.8	120.00±1.6	0.93	0.360
Diastolic BP (mmHg)	80.32±1.3	80.32±1.2	0.00	1.000

*Values are mean±SEM (Standard error of mean), n=number of subjects, t=Student's t-test, p=significance level, *=significant at p<0.05, SBP=Systolic blood pressure, DBP=Diastolic blood pressure, µg/dL=micrograms per decilitre, nmol/L=nanomole per litre, pmol/L=picomole per litre, mmHg=millimetre mercury.*

Table 3: Comparison of age, anthropometric indices and blood pressure measurements in women with and without breast cancer.

Variable	Cases (n=85)	Control (n=84)	t	*P*
Age (years)	48.3±1.3	48.45±1.27	- 0.07	0.941
Toxic metals				
Lead (µg/dL)	5.5±0.2	1.8±0.0	24.17	<0.001*
Cadmium (µg/dL)	0.04±0.0	0.01±0.0	24.60	<0.001*
Arsenic (µg/dL)	0.3±0.0	0.04±0.0	23.21	<0.001*
Hormones				
Oestradiol (pmol/L)	344.8±31.9	307.8±34.7	0.79	0.433
Progesterone (nmol/L)	8.6±1.8	5.9±1.4	1.17	0.245
FT_4 (pmol/L)	17.8±0.4	14.7±0.3	6.37	0.000*
TSH (mIU/L)	1.7±0.1	1.4±0.1	1.88	0.062
Anthropometric indices				
Waist circumference (cm)	89.9±1.1	82.6±1.2	4.54	<0.001*
Hip circumference (cm)	101.8±1.1	98.5±1.0	2.21	0.028*
Weight (kg)	69.2±1.4	62.1±1.1	3.98	<0.001*
Height (m)	1.63±0.0	1.58±0.0	4.88	<0.001*
Body mass index	26.1±0.5	24.9±0.4	1.83	0.070
Waist hip ratio	0.9±0.0	0.8±0.0	4.86	<0.001*
Waist height ratio	55.3±0.7	52.4±0.7	2.81	0.006*
Blood pressure				
Systolic BP(mmHg)	122.7±1.1	119.4±1.0	2.22	0.028*
Diastolic BP (mmHg)	81.7±0.9	80.7±0.8	0.81	0.418

*Values are in mean±SEM (Standard error of mean), n=number of subjects, t=Student's t-test, p=significance level, *=significant at p<0.05, SBP=Systolic blood pressure, DBP=Diastolic blood pressure, µg/dL=micrograms per decilitre, nmol/L=nanomole per litre, pmol/L=picomole per litre, mmHg=millimetre mercury*

Table 4: Correlation of biochemical parameters, anthropometric indices and blood pressure in women with breast cancer

Index	Index	r	*P*
Progesterone	Oestradiol	0.692	0.000
	Diastolic blood pressure	0.218	0.045
Oestradiol	Diastolic blood pressure	0.214	0.050
FT_4	Arsenic	-0.346	0.001
TSH	Height	0.324	0.002
Lead	Cadmium	0.953	0.000
Cadmium	Diastolic blood pressure	0.238	0.028
Waist circumference	Hip circumference	0.803	0.000
	Waist hip ratio	0.473	0.000
	Waist height ratio	0.933	0.000
	Body weight	0.372	0.000
Hip circumference	Waist height ratio	0.741	0.000
	Body weight	0.476	0.000
Waist hip ratio	Waist height ratio	0.455	0.000
Waist height ratio	Height	-0.391	0.000
Systolic blood pressure	Diastolic blood pressure	0.426	0.000
Height	Body weight	0.409	0.000

r= Pearson correlation coefficient, P= probability, p<0.05=significant

Table 4 shows the correlation of biochemical parameters, anthropometric indices and blood pressure in women with breast cancer. Progesterone positively correlated with oestradiol and diastolic blood pressure (p<0.05). Oestradiol also positively correlated with diastolic blood pressure (p<0.05).

Table 5: Correlation of hormones, endocrine disruptors, anthropometric indices and blood pressure in women without breast cancer

Index	Index	r	*P*
Progesterone	Oestradiol	0.538	0.000
Oestradiol	Waist circumference	-0.368	0.001
	Hip circumference	-0.289	0.008
	Waist hip ratio	-0.298	0.006
	Waist height ratio	-0.286	0.009
	Height	-0.280	0.010
	Body weight	-0.263	0.016
Lead	Cadmium	0.869	0.000
Arsenic	Waist circumference	0.253	0.020
	Waist hip ratio	0.322	0.003
	Waist height ratio	0.266	0.014
Waist circumference	Hip circumference	0.820	0.000
	Waist hip ratio	0.725	0.000
	Waist height ratio	0.957	0.000
	Body weight	0.628	0.000
Hip circumference	Waist height ratio	0.766	0.000
	Body weight	0.657	0.000
Waist hip ratio	Waist height ratio	0.715	0.000
	Body weight	0.298	0.006
Waist height ratio	Body weight	0.505	0.000
Diastolic blood pressure	Height	-0.265	0.015
	Body weight	0.242	0.027
Height	Body weight	0.398	0.000

r= Pearson correlation coefficient, P= probability, $p<0.05$=significant

FT_4 inversely correlated with arsenic ($p<0.05$). TSH positively correlated with height ($p<0.05$). Lead correlated positively with cadmium, cadmium correlated positively with diastolic blood pressure ($p<0.05$). There were positive correlations between waist circumference, hip circumference, waist hip ratio, waist height ratio, and body weight ($p<0.05$). Hip circumference positively correlated with waist height ratio and body weight ($p<0.05$). Waist hip ratio positively correlated with waist height ratio, while waist height ratio inversely correlated with height ($p<0.05$). Height correlated positively with body weight ($p<0.05$). Systolic blood pressure positively correlated with diastolic blood pressure ($p<0.05$).

Table 5 shows the correlation of biochemical parameters, anthropometric indices and blood pressure in women without breast cancer. Progesterone correlated positively with oestradiol. Oestradiol correlated inversely with waist circumference, hip circumference, waist hip ratio, waist height ratio, height ad body weight $p<0.05$. Lead positively correlated with cadmium ($p<0.05$). Arsenic positively correlated with waist circumference, waist hip ratio and waist height ratio ($p<0.05$). Waist circumference positively correlated with hip circumference, waist hip ratio, waist height ratio and body weight ($p<0.05$). Hip circumference positively correlated with waist height ratio and body weight ($p<0.05$). Waist hip ratio positively correlated with waist height ratio and body weight ($p<0.05$). Waist height ratio positively correlated with body weight. Height positively correlated with body weight ($p<0.05$). Diastolic blood pressure inversely correlated with height but positively with body weight ($p<0.05$).

Discussion

Breast cancer incidence in women has been related to industrialization consequent upon the widespread contamination of the soil, air and water by the toxic metals [29]. In this present study, serum Cd level was significantly higher in premenopausal cases, postmenopausal cases and cases compared with their respective controls ($p<0.05$). The ability of cadmium to induce cell proliferation, differentiation, apoptosis and signal transduction by enhancement of protein phosphorylation, activation of transcription and translation factors suggests its ability to induce breast cancer [30]. Moreover, Cd has the potential to disrupt endocrine function by behaving like sex hormones

[31]. At low concentrations, the metal mimics the effects of oestradiol and binds with high affinity to the hormone-binding domain of oestrogen receptor alpha (ERá). This binding involves several amino acids, suggesting that Cd activates the receptor through the formation of a complex with specific residues in the hormone-binding domain [32]. Hypermethylation and repression of DNA repair genes appear to be an early signature of cadmium-induced cancer and may constitute part of the mechanisms by which the toxicant induces tumourigenesis [33].

Lead is of concern due to its wide use [34]. However, results of epidemiologic studies investigating the association of Pb with cancers are inconsistent and vary according to the type of cancers reported [35, 24]. Direct DNA damage as a result of oxidative stress, clastogenicity, inhibition of DNA synthesis or repair have been reported as the mechanisms of Pb carcinogenicity [36, 3]. In this present study, serum Pb was significantly higher in premenopausal cases, postmenopausal cases and cases when compared with their corresponding controls ($p<0.05$). This is consistent with the findings of Siddiqui et al. [37] in which blood Pb level was significantly higher in breast cancer cases than controls. There are reports that Pb adversely affects steroidogenesis by substituting for zinc in the DNA binding zinc (Zn^{2+})-finger motif of steroidogenic enzymes. These enzymes are Steroidogenic Acute Regulatory Protein (StAR), Cytochrome P450 side chain cleavage enzyme (CYP450cc) and 3 beta hydroxysteroid dehydrogenase (3â HSD). This results in decrease in the expression of these enzymes [38].

Arsenic exposure constitutes one of the most wide-spread environmental carcinogens and is associated with increased risk of different types of cancers [39] However, few studies have focused on the association of environmental exposure to arsenic and breast cancer risk. Prior to this study, information on the association of arsenic with breast cancer in sub-Saharan Africa is sparse. In this present study, the serum level of arsenic was significantly higher in premenopausal cases, postmenopausal cases and cases than their respective controls ($p<0.05$). Transcription factors in human MDA-MB-435 breast cancer and rat H4IIE hepatoma cells were reportedly sensitive to low dose arsenic [40]. Arsenic is thought to induce carcinogenicity by inducing DNA hypomethylation leading to aberrant gene expression [41, 42] or by DNA methylation silencing genes associated with controlling tumourigenesis [43]. Arsenic competes with DNA methyl transferase genes (DNMT) for S adenosylmethionine (SAM), potentially limiting the availability of SAM to be used by DNMT to catalyze methylation of CpG. This could result in hypomethylation and reactivation of silenced tumour suppressor genes [44, 45]. Altered histone modification associated with arsenic-induced gene expression in carcinogenesis has been suggested [33].

Our observations show the contribution of environmental factors/endocrine disruptors-lead, cadmium and arsenic to breast cancer. Our previous study showed the contribution of adiposity and other endocrine disruptors-polychlorinated biphenyls and bisphenol-A levels to breast cancer in Nigeria [26]. In this present study, WC, HC, height, weight, WHR and WHtR were significantly higher in cases when compared with controls ($p<0.05$). Height and weight were significantly higher in both pre and postmenopausal women compared with their respective controls ($p<0.05$). This is in tandem with reports of an association between higher body weight and increased breast cancer risk in postmenopausal women [46]. Although, height has also been linked to increased breast cancer risk in postmenopausal women, this association is not clear in premenopausal women [48, 49]. Ogundiran et al. [47] demonstrated that height was a significant risk factor for female breast cancer in both pre and postmenopausal women. The underlying mechanism could be that childhood energy balance is associated with mammary gland mass and increased insulin-like growth factors [50]. Attained height is determined by genetic makeup and environmental factors, including energy intake during childhood and adolescence. In societies with an insufficient food supply, caloric intake plays a more important role in determining height than in societies with an abundant food supply [50]. Thus, energy intake in earlier life may play an important role in breast carcinogenesis.

Body mass index is a measure of overall adiposity. It has been shown to have a significant role in the identification of obese and overweight individuals [51]. There was no difference in the BMI between cases and controls ($p<0.05$) in this study. Similar observation was observed in the pre and postmenopausal cases compared with their respective controls. Charles-Davies et al. [9] reported that the metabolic complications of overweight and obesity could be more related to the location of body fat rather than to the amount of total body fat which is measured by BMI. Visceral obesity has been strongly associated with metabolic dysfunctions including metabolic syndrome and type 2 diabetes mellitus and could predispose individuals to breast

cancer [10, 52]. In this present study, determinants of visceral obesity (WC, WHR, WHtR and subcutaneous obesity (HC) were significantly higher in only premenopausal cases than controls ($p<0.05$). Our observations corroborate the study of Fagherazzi *et al*. [53]. The association of visceral and subcutaneous obesity with breast cancer in premenopausal cases is therefore suggested. However, similar observations were made in a previous study in premenopausal apparently healthy Nigerian women with metabolic syndrome without breast cancer [52]. It is therefore possible that visceral adiposity and subcutaneous adiposity alone may be not be involved in premenopausal breast carcinogenesis.

Arsenic correlated positively with waist circumference, waist hip ratio and waist height ratio in controls in this study**.** This could be explained by the fact that arsenic is lipophilic and probably have preference for subcutaneous fat. Studies have shown that obesity is marked by alteration in the production of adipocytokines; leptin and adiponectin. Increased leptin levels and decreased adiponectin levels promote breast carcinogenesis [53]. Leptin is strongly angiogenic and may increase tumour angiogenesis by directly acting on the endothelium or by increasing local vascular endothelial growth factor (VEGF) secretion [54, 55]. Studies in Ibadan showed elevated leptin levels in apparently healthy premenopausal women with metabolic syndrome compared with those without metabolic syndrome. Leptin levels were similar in both pre and postmenopausal women with metabolic syndrome [51]. Earlier study showed that elevated levels of leptin in individuals with metabolic syndrome might reflect adiposity and could be a compensatory mechanism for maintaining weight/fat loss and blood pressure [51]. An inverse correlation was observed between FT_4 and As in cases in this study. This suggests the possible interference of thyroid hormones by arsenic in women with breast cancer. This could be due to the binding of As to the thyroid hormone receptors which blocks the binding of the thyroid hormones.

In this present study, the mean values of SBP and DBP in the cases and controls reflect normal blood pressure. However, SBP was significantly higher in cases and premenopausal cases than their respective controls ($p=0.028$; $p=0.042$, respectively). This might reflect the mild increase in visceral obesity in premenopausal cases compared with their controls. Reports of the relationship of steroid hormones with blood pressure have not been clearly defined [56, 57]. Positive correlation was observed between oestradiol, progesterone and diastolic blood pressure in cases in this study. Oestradiol in contraceptives may increase salt and water retention [58]. Moreover, progesterone has a tendency to antagonize the actions of aldosterone, thereby supporting an increase in blood pressure [58]. The positive correlation observed between cadmium and diastolic blood pressure in the cases was in tandem with the observation of Gallagher and Meliker [59]. The inhibition of endothelial nitric oxide synthase protein in blood vessels, which suppresses acetylcholine-induced vascular relaxation to induce hypertension is a possible mechanism [60].

Conclusion and recommendation

Findings in this study implicate Cd, Pb and As in breast carcinogenesis. Therefore, routine screening for these toxic metals as well as reduction of environmental pollution by advocacy and education may significantly reduce the incidence of the disease.

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