

Lead Exposure: A Contributing Cause of the Current Breast Cancer Epidemic in Nigerian Women

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Abstract Breast cancer incidence in Nigerian women has significantly increased during the past three decades in parallel with the rapid industrialization of that country. This suggested that the associated widespread contamination of the soil and of the water supplies by lead (Pb) and other industrial metals was a major contributing cause. Because of its many domestic, industrial, and automotive uses, Pb is of particular concern as it has been shown to promote the development of mammary tumors in murine mammary tumor virus-infected female C3H mice at levels as low of 0.5 ppm Pb in the drinking water. Lead belongs to the group of selenium-antagonistic elements that interact with selenium (Se), abolishing its anti-carcinogenic effect. Lead on chronic, low-level exposure in addition also accelerates tumor growth rates. Higher levels of Pb were found in blood and head hair samples of newly diagnosed patients with breast cancer, all with infiltrating ductal carcinoma, the most common form of breast cancer in Nigeria, seen at Obafemi Awolowo University, than in cancer-free controls from the same area. Evidence for interactions between Pb and Se was obtained from blood, hair, and tumor biopsy tissue analyses. Furthermore, the Pb levels in hair samples of the patients were directly correlated with the volumes of their tumors, in accord with the tumor growth-promoting effects of Pb. Conversely, Se levels in hair and blood were inversely correlated with the tumor volumes, consistent with the anti-proliferative effects of Se. Several other elements, e.g., Cd, Hg, Cr, Sn, and As, were detected in the scalp hair of the patients and the controls, although at significantly lower levels than those of Pb. However, correlation calculations revealed them also to interact with Se, suggesting that only a fraction of the Se in organs and tissues is actually present in bioactive forms. In metal-exposed subjects, a state of latent Se deficiency may exist, resulting in depressed immune functions and increased cancer susceptibility. Evidence is presented to show that Pb and other metals also interact with iodine, another vitally

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important essential trace element believed to protect against breast cancer development. Public health programs aiming at lowering the breast cancer risk of Nigerian women thus will have to include effective measures to protect the population from exposures to Pb and other industrial metals that are presently contaminating the environment and the water supplies.

Keywords Breast cancer · Lead · Cadmium · Tin · Chromium · Arsenic · Selenium · Iodine · Nigeria · Metal pollution

Introduction

Breast cancer ranks first among cancers occurring in women and second only to lung cancer as a cause of death in women worldwide [1]. Whereas breast cancer incidence is higher in the developed countries than in the developing countries, its incidence in the latter is increasing. In Nigeria, in particular, the incidence increased from 13.8–15.3/100,000 women in 1976 to 33.6/100,000 in 1992 and reached 116/100,000 in 2001, and is now the leading cause of cancer deaths among Nigerian women [2]. The causes of this alarming trend are not known. As changes of demographic profile, the acquisition of Western lifestyles, obesity, and altered socioeconomic status alone cannot explain this development, we hypothesized that the contamination of the soil and the water supplies by toxic metals during the period of industrialization could be a major contributing cause. Elements of interest within this context are lead (Pb), chromium (Cr), cadmium (Cd), zinc (Zn), tin (Sn), and arsenic (As). However, the degree to which these elements might increase breast cancer risk depends yet on another element, selenium (Se), a nutritionally essential trace element whose anticarcinogenic properties have been demonstrated in numerous animal tumor model systems, and there is evidence that cancer mortalities in different countries are inversely associated with the dietary intakes of this element [3]. However, Se also interacts with other elements, mostly toxic heavy metals, *in vivo*. These interactions are part of natural metal detoxification processes, but result in the metabolic inactivation of Se and at sufficiently high exposure levels may over time produce a state akin to Se deficiency. In studies with female C3H mice carrying the murine mammary tumor virus (MMTV), Pb at subtoxic levels was shown to abolish the cancer-protecting effect of Se at chronic exposure levels as low as 0.5 ppm in the drinking water [4]. In Nigeria, the dietary Se intakes of the population are generally adequate [5], but Pb is an element of major concern as its many industrial and domestic uses, the presence of tetraethyl lead in gasoline, and the largely uncontrolled dumping of Pb-containing residues have severely contaminated the soil and the water supplies in many parts of the country [6, 7]. Accordingly, a large segment of the population of Nigeria is chronically Pb-exposed, including children. In one study of 218 children aged 6–35 months, 70% had blood lead levels in excess of 10 $\mu\text{g}/\text{dL}$ [8]. In adults, occupationally Pb-exposed auto-mechanics exhibited mean blood and hair Pb levels of $48.50 \pm 9.08 \mu\text{g}/\text{dL}$ and $17.75 \pm 5.16 \mu\text{g}/\text{g}$, respectively [9], but Pb levels of unexposed controls from the same city (Abeokuta, in SW Nigeria) were elevated, with $33.65 \pm 10.09 \mu\text{g}$ Pb per deciliter in blood and $14.30 \pm 5.90 \mu\text{g}$ Pb per gram in hair. Evidently, Pb pollution is so widespread that unexposed controls are difficult to find. The dangers of chronic Pb exposure are well recognized. Thus, a 1998 study found the immune status of occupationally Pb-exposed Nigerian workers to be decreased, and its author pointed out that this may increase their susceptibility to infectious diseases, inflammatory disorders, and cancer [10]. However, since other metals may have similar effects and increase breast

cancer risk, we decided to perform multi-element analyses of whole blood and scalp hair from breast cancer patients and from cancer-free controls, all from Ile-Ife, Osun State, Nigeria. In addition, element analyses were also obtained from breast biopsy samples of these patients. While our study was in progress, a report on trace element levels in the plasma and sera of Nigerian women from the Ibadan area with and without breast cancer was published [11]. These authors found lower Cu, Zn, and manganese (Mn) levels in the patient sera, but Pb and a number of other elements were not significantly elevated compared to their controls. Osun, Ondo, and other nearby states are in a region where almost 100% of the surface water-bearing wells have recently been reported to contain Pb, Ni, and Cr at levels exceeding the WHO and Nigeria Federal Ministry of Environment guidelines [12]. Furthermore, as plasma or serum contain these elements in significantly lower amounts, we determined them in whole blood, and since blood reflects only the most recent exposures, hair samples of the cases and controls were analyzed as well to obtain a record of the element exposures during the past several months. A total of 34 elements were analyzed in hair, which included iodine (I), a nonmetallic essential element believed to have protective functions against breast cancer development [13–15].

Materials and Methods

Participants

Twelve newly diagnosed breast cancer patients were recruited from the Surgery Clinics of the Obafemi Awolowo University Teaching Hospital Complex, Ile-Ife, Osun State, Nigeria.

Anthropometric data of the patients were measured and recorded. Also obtained was information on the current drug treatment. The hair treatment agents were recorded for all the patients and controls. Independent tests revealed none of the agents to contain lead. Table 1 lists socio-demographic data as obtained from completed questionnaires: type of tumor, tumor stage, histological characteristic of the tumors, and the body mass indexes (BMIs) as calculated from the heights and weights of the patients using the formula: $BMI (kg/m^2) = Weight (kg)/Height^2 (m^2)$.

The control subjects consisted of 12 aged-matched apparently healthy women, mostly members of the staff and students of the Obafemi Awolowo University Teaching Hospital Complex, Ile-Ife, Nigeria. To ensure uniformity, all patients and controls chosen were from the same ethnic background (Yoruba) and living in the southwestern part of Nigeria, as determined from their responses to questionnaires and clinical investigations. Excluded were pregnant or lactating women, women on hormonal therapy, women with inflammatory conditions, women on regular hematinics or taking supplements containing selenium, and women with diabetes mellitus, hypertension, hepatitis, tuberculosis, and jaundice.

Blood, Tumor Tissue, and Hair Samples

Samples of fasting blood of the patients and the controls were collected and were stored in tubes. Samples of scalp hair from the patients and the controls were taken using Teflon scissors and placed into polyethylene bags provided by the laboratory performing the determinations. Breast biopsy samples of the same patients were collected and prepared for trace element analysis using formalin as a preservative. The analyses of all samples, including the formalin solution, were performed by state-of-the-art inductively coupled plasma mass spectrometry methods [16].

Table 1 Patient Data

No.	Age	Age at menarche	Marital status/parity	SES	BMI	Tumor size (cm)	TNM/His ^a	Stage	Nottingham score
1	80	20	M/8	2	18.8	6×5×2	T3N1M0/IDC	2	3
2	68	16	M/8	1	23.6	7×4×3	T4N1M0/IDC	3	3 ³
3	49	13	M/4	2	28.3	5×3×2	T2N1M0/IDC	2	2
4	40	15	M/5	1	25.6	12×8×5	T4N2M1/IDC	4	3 ³
5	58	15	M/0	1	25.0	10×8×6	T4N2M0/IDC	3	3
6	60	19	M/7	2	24.6	4×3×3	T2N1M0/IDC	1	3
7	51	20	M/3	2	28.7	8×5×4	T3N1M0/IDC	2	3
8	40	20	M/4	2	34.8	10×8×8	T4N2M0/IDC	3	3
9	62	16	M/5	3	29.1	12×10×10	T4N1M0/IDC	3	3
10	40	16	M/0	1	25.3	6×4×3	T3N0M0/IDC	2	3
11	44	14	M/4	1	34.8	10×8×6	T4N2M0/IDC	3	3 ^b
12	45	15	M/5	1	25.5	12×8×8	T4N1M1/IDC	4	3 ^b

IDC infiltrating ductal carcinoma

^a SES socio-economic status: 1 = low, 2 = medium, 3 = high

^b Expired

Results

The histologically confirmed type of breast cancer of all patients of the present study was invasive ductal carcinoma (IDC), the most common form of breast cancer in Nigeria. The tumors of all patients were locally advanced, as assessed in terms of their TNM stage and their Nottingham's scores. The ages of the patients, their ages at menarche, marital status, parity, socioeconomic status, BMI, tumor size, tumor stage, number of nodes and histology (TNM/His), disease stage, and their Nottingham scores are given in Table 1. The ages ranged from 40 to 80 years, and the median was 50 years. Their median BMI was 25.6 and their median age at menarche 16.0 years. Most of the patients were multigravida and on average breastfed each child for over 1 year. Socioeconomic status (SES) of the patients was assessed on annual income and was numerically expressed as 1 for the low, 2 for the medium, and 3 for the high-income categories. The SES of six patients was low, of five patients medium, and of one high. While this study was in progress, four patients expired. Death of these patients was associated with low SES ($P<0.02$), high tumor stage ($P<0.01$), and large tumor volume ($P=0.05$), but not with age ($P=0.32$) and the BMI ($P=0.78$).

Elements in Whole Blood and Hair Samples

Average concentrations and ranges of elements determined in whole blood and in hair of the patients and controls, with calculated Student's *t* test *P* values and laboratory reference ranges are given in Tables 2 and 3. Table 4 lists elements determined in the breast tumor biopsy samples by us and by other authors. Although the samples were analyzed for a total of 20 elements, the results for only 11 elements are given since the remaining nine elements, i.e., Sb, Be, Bi, Pt, Tl, Th, U, Hg, and W, were either present in amounts below the detection limit or absent. Based on the results in Table 4, the tumors of our patients contained comparable amounts of Zn, Cu, Fe, and Ni and more Cr, As, and Se than that reported by Risk and Sky-Peck [17]. Our mean amount of Pb was lower but essentially the

Table 2 Blood Analysis Results

Elements and units	Control		Cases		<i>P</i> value ^a	Reference range
	Mean	Range	Mean	Range		
Cu (µg/dL)	98.9	76–145	132.5	105–157	<0.001	61–122
Zn (µg/dL)	617	410–760	627.6	491–777	0.15	420–810
Pb (µg/dL)	5.0	1.8–8.5	6.1	2.2–14.0	<0.01	<3.5
Se (µg/L)	303	150–462	291	181–601	0.79	130–340
Cd (µg/L)	1.7	1.4–2.1	1.5	0.4–1.9	0.85	<4.0
Hg (µg/L)	1.9	0.6–3.6	2.4	0.9–5.2	0.11	<5.0
As (µg/L)	6.8	4.0–12	7.6	3.4–16	0.11	<20
Mn (µg/L)	10.4	6–19	9.1	6.0–15	0.56	6–19
Sr (µg/L)	34.5	3.8–67	33.7	19–64	0.10	8–38
Ca (mg/dL)	5.8	5.3–5.6	5.9	5.5–6.4	0.31	4.4–6.9
Mg (mg/dL)	3.4	2.9–4.2	3.6	2.9–4.2	0.65	2.8–4.4
Li (µg/L)	0.76	0.5–1.7	0.95	0.4–1.8	<0.01	0.3–20
Co (µg/L)	0.53	0.2–0.9	0.42	0.2–1.1	0.91	<4.0
Zn/Cu	7.6	5.4–8.6	5.2	3.2–7.6	0.004	– ^b
Ca/Mg	1.70	1.5–2.1	1.63	1.4–2.2	0.60	– ^b

^a Student's *t* test^b Reference range not available

same as the median level reported by Ionescu et al. [18, 19]. The median amounts of the elements measured by the latter authors were comparable or within range to ours, but their value for Al given in [18] was significantly lower.

Discussion

Associations with Demographic Parameters

Partitioning of the patients with respect to their SES revealed low SES associated with higher tumor stage ($P<0.0008$) and with larger tumor volumes on admission ($P<0.03$), suggesting that the women of low SES sought treatment later than women of the medium or high socioeconomic classes. This conclusion is supported by the fact that the four patients who expired while this work was in progress all belonged to the lowest socioeconomic class, were in advanced tumor stages, and had large tumors. Low SES also was associated with earlier menarche ($P<0.05$), but not with parity ($P=0.38$) or BMI ($P=0.89$). Among trace elements, low SES was associated with low I levels in hair ($P=0.01$), high Cu concentration in blood ($P=0.020$), and low Zn/Cu ratios in blood ($P=0.02$), both reflecting more advanced disease.

Associations with Individual Elements

Lead According to Table 2, the means and the ranges of blood lead levels of the controls and cases were 5.0, 1.8–8.5 and 6.1, and 2.2–21 µg/dL, respectively. Both means are above the laboratory reference value for Pb of 3.5 µg/dL [15], and the difference of the means between cases and controls is statistically significant ($P<0.01$). It should be noted that the

Table 3 Elements in Scalp Hair of Cases and Controls (Unit: mcg/g)

Elements	Control		Cases		P value ^a	Reference range
	Mean	Range	Mean	Range		
Pb(all)	251	0.76–2900	497	5–5000	.75	<1
Pb ^b	10.3	0.76–26	23.8	5–67	.19	<1
Cd	0.32	0.004–0.87	0.45	0.03–3.6	.009	<.10
Hg	0.40	0.02–1.2	0.67	0.06–2.74	.53	<1.1
As	0.09	0.02–0.18	0.08	0.004–0.18	.28	<.30
Cu	40.1	9.7–110	48.41	9.6–110	.05	12–35
Zn	617	410–760	627	491–777	.15	140–220
Se	0.886	0.48–1.34	0.727	0.51–1.1	.04	.95–1.7
Mn	23.7	1–170	15.8	4.8–36	.08	.15–.65
Sr	9.4	1.7–27	7.7	2.3–25	.03	.5–7.6
Ca	2530	369–865	1617	360–5570	.01	300–1200
Mg	477	57–2600	227	55–990	.31	35–120
Li	0.053	.4–1.7	0.046	0.5–1.8	.09	.007–.023
Co	1.74	0.03–15.5	0.93	0.13–4.5	<.01	.013–.050
I	1.85	0.32–5.9	1.27	0.2–3.32	.96	.25–1.3
S	35336	(2–4.5) × 10 ⁴	36437	(2–4.5) × 10 ⁴	.57	(4.4–5.2) × 10 ⁴
P	166	91–199	149	90–202	.56	160–250
Ag	0.10	0.01–0.32	0.067	0.01–0.32	.096	<.15
Fe	96.9	31–300	148	30–347	.17	5.4–14
Cr	1.10	0.62–2.1	0.96	0.45–1.45	.36	.20–.40
V	0.27	0.082–0.78	0.35	0.078–0.78	.98	.018–.065
Mo	0.12	0.01–0.46	0.23	0.026–1.0	.81	.028–.056
B	4.55	0.84–19	3.35	1.0–11	.48	.30–2.0
Na	852	24–4200	510	19–4200	.005	12–90
K	417	12–1100	393	25–970	.19	8–38
Ba	18.6	1.4–130	14.2	4.2–41	.86	.26–3.0
Be	0.026	0.001–0.25	1.94	0.01–25	.93	<0.020
Bi	0.49	0.017–3.8	0.23	0.02–1.7	.38	<2.0
Ni	1.98	0.08–7	1.91	0.2–7.8	.09	<0.40
Tl	0.49	0.001–3.2	0.01	0.003–0.06	.42	<0.010
Th	0.010	0.003–0.026	0.019	0.003–0.057	.26	<0.005
U	0.029	0.003–0.072	0.53	0.017–0.15	.15	<0.060
Ti	1.74	3.7–21	2.4	0.52–5.9	.31	<1.0
Sn	15.3	0.1–120	0.2	0.38–120	.46	<0.30
Al	39.6	10–90	69.7	13–150	.06	<7.0
Zn/Cu	8.21	1.2–16.3	4.58	1.0–8.1	.34	4–20
Ca/Mg	8.35	1.95–17.4	8.91	3.3–16.8	.28	4–30
Ca/P	17.3	2.1–95	8.91	2.5–35	.001	1–12
Na/K	1.46	0.41–4.33	1.04	0.32–4.33	<.001	0.5–10
Zn/Cd	688	296–999	653	564–999	.13	>800

^a Student t-test. ^b High Pb outliers (1200–5000 mcg Pb/g) omitted

Table 4 Elements in Breast Tumor Tissue Samples (mcg/g dry wt.)

	This work		Rizk and Sky-Peck [17]	Ionescu et al. [18, 19]	
	Median	Range	Median	Median	
Pb	0.11	0.022–0.21	1.55±1.24	0.10	0.009–0.98
Cr	0.91	0.55–1.28	0.49±0.80	0.82	0.31–5.98
Cd	0.14	0.05–0.36	ND	0.042	0.009–0.55
As	0.077	0.032–0.11	0.13±0.09	0.03 ^a	
Zn	22.8	5.95–54.9	68.1±26.0	17.1	1.3–97.9
Cu	2.79	1.0–5.95	21.0±10.7	0.92	9.32–44.7
Se	0.96	0.19–1.60	1.02±0.43	ND	
Fe	91.1	19.6–196	238.5±113.0	53.2	14.7–205.9
Al	2.11	0.59–4.35	ND	0.17 ^a	
Ni	0.100	0.014–0.32	1.27±0.59	0.99	0.407–3.36
Sn	0.022	0.014–0.060	ND	0.044 ^a	

ND not determined

^aRange not given

patient with the highest blood Pb level, 14 µg/dL (no. 12 in Table 1), was with stage 4 breast cancer and spinal metastases, the most seriously ill, and has since died of her disease.

Pb in blood was directly correlated with Pb in tumors ($P=0.05$), directly with Se in blood ($P=0.02$), and inversely correlated with hair Se ($P=0.04$). Pb in tumors, on the other hand, was inversely associated with blood Se ($P=0.04$). These results indicate that Pb interacts with Se in vivo.

The mean Pb in hair, after elimination of the high outliers with levels of 1,200–5,000 µg Pb per gram, was 23.6 µg/g, more than twice higher in the cases than the 10.3 µg/g of the controls (see Table 3). Associations of hair Pb levels of the cases were significantly direct with the BMI ($P=0.0455$), as well with the logs of the tumor volumes ($P=0.0482$), and inverse with the hair Zn ($P=0.0174$) and blood Se values ($P=0.05$).

On the other hand, hair Pb was directly correlated with hair iodine ($P=0.034$), which is to be expected as Pb has a high affinity for iodine and there is evidence of interactions between the two elements in vivo.

Some of the same elements were previously reported by Nnorom et al. [20] from analyses of hair samples collected in three industrialized and non-industrialized towns in southeastern Nigeria. These authors found that 89% of their study subjects had hair Pb levels >30 µg/g, 20% levels >110 µg/g. Of our patients, four (33%) had Pb values >30 µg/g, among them one with Pb levels reaching 1,200 µg/g and another with 5,000 µg/g. Only one of our controls exhibited a similarly high Pb value, 2,900 µg/g; the Pb levels of the remaining controls were <30 µg/g.

Selenium Blood Se was inversely correlated with the logarithms of the tumor volumes ($P=0.03$), suggesting that Se inhibits tumor growth.

Blood Se was directly correlated with Se in hair ($P=0.05$); the association with Se in tumors was direct but statistically insignificant ($P=0.07$). Hair Se was directly correlated with age ($P=0.0057$) and with parity ($P=0.036$) and inversely correlated with BMI ($P=0.037$). As to the interactions of Se with Pb in blood, tumors, and hair, see the section on 'lead' above. In addition, blood Se was inversely correlated with Zn in blood ($P=0.004$), Sn

in hair ($P=0.027$), and Mo in hair ($P=0.032$) and directly correlated with Zn in tumors ($P=0.002$). Direct associations of hair Se with hair As in patients and in controls were significantly direct, with $P<0.005$ and $P<0.02$, respectively. The associations of Se with Pb, Zn, Cr, Sn, and As are not unexpected as these elements are known to interact with Se and to possess Se-antagonistic properties. In experiments with female C3H mice infected with the MMTV, the exposure of the animals to low levels of Pb, Zn, Cr, and As in the supply water abolished the cancer-protecting effects of Se and stimulated tumor growth [4].

Cadmium Cd has also been shown to interact with Se in vivo and to abolish the cancer-protecting effects of Se in the MMTV-infected mice [21], and a statistically significant direct association between the estimated dietary Cd intake parameters and the breast cancer mortalities in different countries has been reported [22]. In our patients, blood Cd levels were inversely correlated with those of Se, but as blood Cd concentrations were low, this association was only of borderline statistical significance ($P=0.09$). Similarly only of borderline statistical significance ($P=0.06$) was the direct interaction of Cd in the tumors with Se levels in tumors. However, although the statistical significance of these associations was low, the evidence suggests that the two elements interact.

As Cd is also known to interact with Zn, the inverse association of Cd in blood with Zn in the tumors with $P=0.0238$ is of interest as it suggests that Cd inhibits the transfer of Zn to the tumor. In the experiments with C3H mice [21], slower tumor growth in the Cd-exposed mice was attributed to the Cd-induced Zn depletion of the tumors. An inverse correlation was also observed between Cd in hair and Zn in hair ($P=0.044$). On the other hand, the hair Cd levels were directly correlated with BMI ($P=0.0017$), suggesting that Cd has metabolic effects resulting in obesity.

Copper Table 2 reveals patients to have higher blood Cu levels ($P < 0.001$) and lower blood Zn/Cu ratios ($P<0.04$) than the controls. Table 3 shows that hair Cu levels of the patients were also significantly ($P=0.05$) higher than those of the controls. The differences between these values reflect physiological responses to the disease process and/or its treatment, as evidenced by the direct correlation of the blood Cu concentrations with the clinically determined tumor stages of the patients ($P<0.05$) and the tumor volumes on admission ($P<0.03$). Cu in the tumors was directly correlated with tumor Se ($P=0.002$) and with tumor Cd and Zn ($P=0.017$ and <0.01 , respectively). In addition, Cu in hair was directly correlated with Cd in tumors ($P=0.017$). Interactions between Cd and Cu are well known [23, 24].

Zinc Zn in the blood of the patients was inversely correlated with blood Se ($P=0.0042$) and also with tumor Cu ($P=0.04$). The tumor Zn concentrations were directly correlated with those of Se in tumors ($P=0.0018$), with Cu in tumors ($P<0.016$), Cr in tumors ($P<0.04$), and with Cd in tumors ($P=0.007$) and inversely correlated with Cd in blood ($P=0.027$). The hair Zn concentrations of the patients were inversely correlated with the Pb hair ($P=0.0174$), the Mo hair ($P=0.0046$), and Cd hair concentrations ($P=0.044$). The blood and hair Zn/Cu ratios were lower than normal in patients than in the controls (see Tables 2 and 3). This is often the case in cancer patients, but is also seen in patients with inflammatory conditions. The blood Zn/Cu ratios were also significantly correlated with SES ($P=0.02$) and inversely correlated with the tumor volume ($P=0.032$) and the tumor stage they were in ($P=0.020$), in accord with their more advanced stage of disease on admission. The blood Zn/Cu ratios were also directly associated with the hair Na/K ratios ($P=0.04$). As these reflect the ratio between intracellular and extracellular potassium and

are controlled by the Na/K-ATPase activity, they are often lower in cancer patients, but this is also seen in other diseases. The hair Zn/Cu ratios showed no significant associations with SES, tumor stage, or the tumor volumes, presumably because the hair Zn/Cu ratios reflect the average value over 3–4 months prior to hair sample collection.

Iodine Hair I was inversely correlated with tumor stage ($P=0.03$) and tumor volume ($P=0.01$), directly correlated with Cu in blood ($P=0.05$), Cu in hair ($P=0.004$), Ni in hair ($P<0.01$), Pb in hair ($P=0.015$), Mo in hair ($P=0.015$), and As in tumor ($P=0.05$) and inversely with Zn in blood ($P=0.006$). These findings are suggestive of an anti-breast cancer effect of iodine [13–15, 25]. However, the present study suggests that the biological effects of iodine are counteracted by a variety of metallic elements.

Other Elements

Tin (Sn) Several of the study subjects exhibited greatly elevated hair Sn levels in patients more than the controls ($P=0.002$, see Table 3). Exposures to Sn are possible as Nigeria is a major producer of this element. We are led to conclude that excessive exposures to tin should be viewed as harmful and should be avoided. In our Sn-exposed patients, hair Sn values were directly correlated with tumor volumes ($P=0.04$) and inversely correlated with the levels of Se in tumors, consistent with the Se-antagonistic properties of Sn [26].

Molybdenum In hair, Mo was associated inversely with Cr in tumor ($P=0.01$) and Se in tumor ($P=0.02$) and directly with Pb in hair ($P=0.006$). These associations suggest a role of Mo in breast cancer, and there is at least one paper which indicates that Mo inhibits *N*-nitroso-*N*-methylurea-induced mammary carcinogenesis in rats [27].

Chromium In hair, Cr was directly correlated with Fe in hair ($P=0.0035$), Fe in tumor ($P=0.004$), Pb in tumor ($P=0.03$), Pb in hair ($P=0.07$) and with As in tumor ($P=0.003$). Coyle et al. [28] showed with univariate analysis that chromium was positively associated with the breast cancer rates in Texas, between 1995 and 2000. As early as in 1977 [22], comparisons of the age-corrected breast cancer mortalities in different countries with the estimated dietary Cr intakes produced statistically significant direct associations suggesting that Cr promotes breast cancer development. More recent studies established that dietary Cr acts as a Se-antagonist, abolishes the anticarcinogenic effects of Se, and promotes tumor growth in MMTV-infected female C3H mice [29].

Boron In hair, boron (B) was directly correlated with Pb in blood ($P=0.006$), As in hair ($P=0.026$), and with lithium (Li) in hair ($P=0.02$). Our study produced no statistically significant direct and possible beneficial effect or inverse association with Se in hair, blood, or tumors of our patients, but the observed direct association with Pb in blood could mean that it has an indirect effect on transport or tissue distribution of the element. Similarly, with seven of our patients, a statistically highly significant inverse association of the B levels in hair with the Cd concentrations in their tumors was obtained ($r^2=0.865$, $P=0.0024$). Otherwise, little is still known about its role in breast cancer except that boric acid has been shown to inhibit cell growth in certain breast and prostate cancer cell lines [30].

Manganese In hair, Mn was significantly correlated with As in tumor ($P=0.032$) and with Pb in hair ($P<0.04$). Mn occurs in manganese superoxide dismutase (MnSOD) which exists

in several genotypes of which some are believed to cause oxidative damage to DNA and increase breast cancer risk [31]. Epidemiologic studies on the MnSOD genotype may contribute to an increased risk of breast cancer in the presence of specific environmental exposures [32].

Lithium In hair, Li was inversely correlated with Cd in tumor ($P=0.005$) and directly with Cr in hair ($P=0.02$), Fe in hair ($P=0.02$), and As in tumor ($P<0.005$). Lithium, which is used to treat bipolar psychiatric disorders, can stimulate proliferation of a number of cells in tissue culture. Proliferation of MCF-7 human breast cancer cells, which also respond to EGF and estrogens, was stimulated by LiCl (1–5 mM) within the concentration range that is encountered during human therapy with lithium. Stimulation of growth was specific for lithium; rubidium, potassium, and sodium showed no such effect [33]. Another study demonstrated that lithium has significant immunoregulatory effects by increasing the production of both pro-inflammatory cytokines (IFN γ , TNF α , and IL-8) and negative immunoregulatory cytokines or proteins (IL-10 and IL-1RA) [34]. In our subjects, the observed association of Li with other elements may be associated with its emerging role as a nutritionally essential element [35]. One of its effects at low nutritional levels is to affect the transport and organ distribution of other elements.

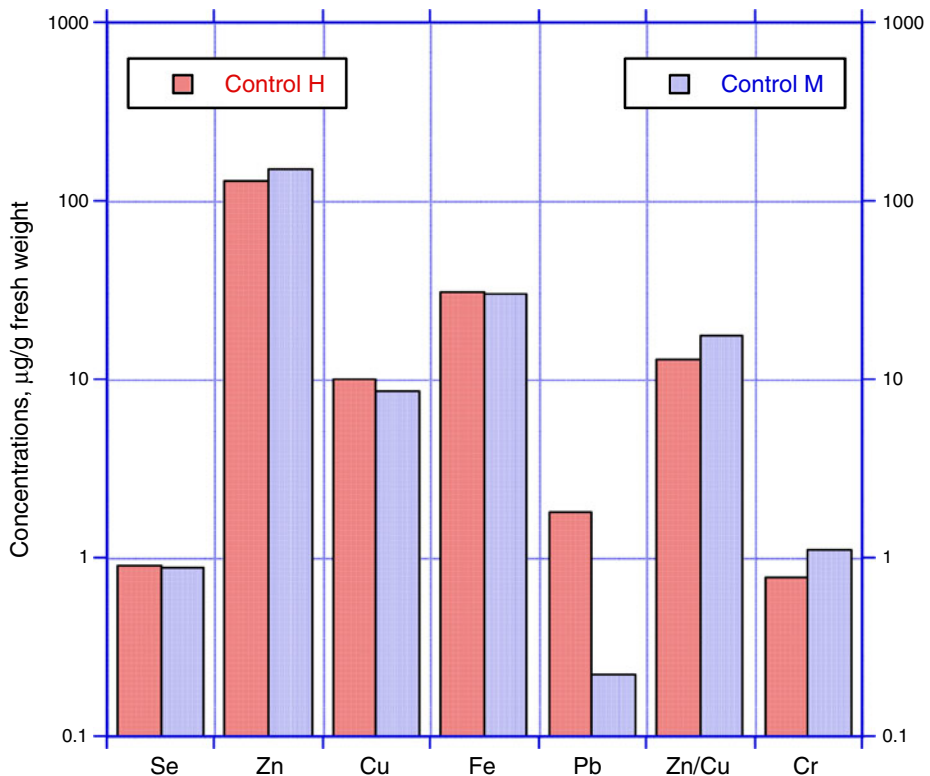


Fig. 1 Comparison of select element concentrations and the Zn/Cu ratios in the hair of a Nigerian control subject ('Control H') with low-Pb exposure with those previously observed [4] in the hair of MMTV-infected female C3H mouse ('Control M')

Alkaline Earth Elements Brief mention is made that the levels of calcium and strontium in hair were lower in the cases than in the controls ($P < 0.01$ and $P = 0.03$), but these differences also cannot be attributed to a specific cancer-related condition. The hair Ca/P ratios of the patients were lower than that of the controls ($P = 0.001$), but were mostly within the reference range of 1–12 irrespective of tumor volume. The one patient with an unusually high Ca/P ratio of 30.4 had stage 1 breast cancer and had recently undergone quadrantectomy followed by radiation and chemotherapy; the combined effects of these treatments may have been responsible for this anomalous result.

Relevance of the Studies with Pb-Exposed MMTV-Infected Mice

In the recently reported study [4] with female MMTV-infected C3H mice, Pb at the level of just 0.50 ppm in the drinking water was shown to abolish the anticarcinogenic effects of 0.65 ppm of Se in the feed. As fragments of a human variant of MMTV have been detected in a significant percentage of human breast cancer tissues [36], the relevance of this tumor model system to human breast cancer is well established. Furthermore, since the exposure level of Pb chosen in that study was of the same order of magnitude as found in some human water supplies, comparisons of the results with the mice [4] with those of Pb-exposed Nigerian women with and without breast cancer are permissible.

Figure 1 shows the observed levels of Se, Zn, Cu, Fe, Pb, Cr, and the Zn/Cu ratio in the hair of lead-unexposed female C3H mouse and of one of our “low-Pb” human controls of the present study. Figure 2 compares the same elements and the Zn/Cu ratio in the hair of a

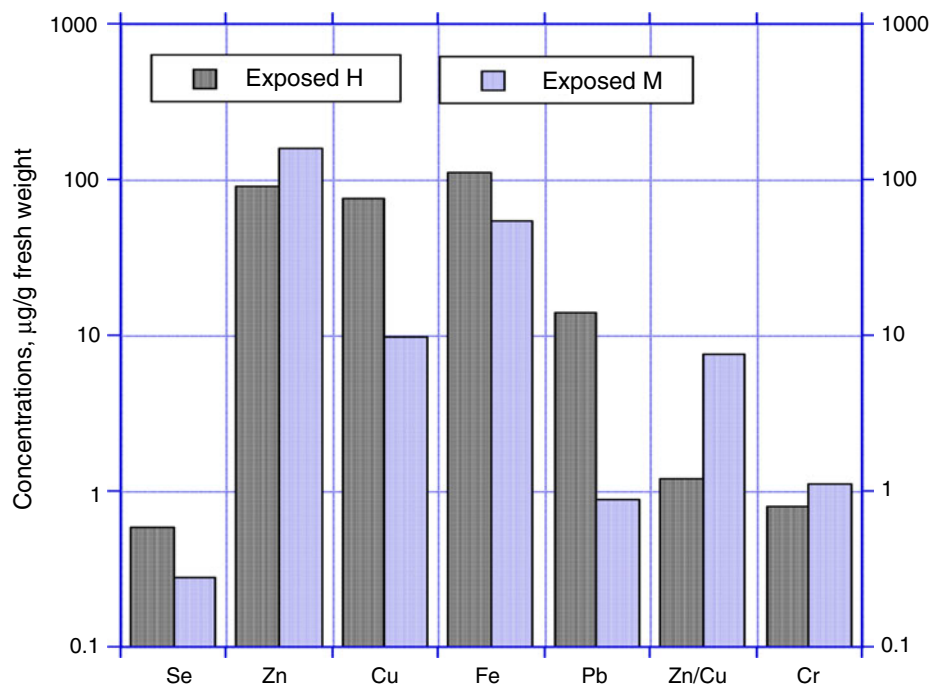


Fig. 2 Comparison of select element concentrations and the Zn/Cu ratios in the hair of a Nigerian control subject with high Pb exposure (‘Control H’) with those previously observed [4] in the hair of MMTV-infected female C3H mouse exposed to 0.5 ppm Pb in the supply water (‘Control M’)

Pb-exposed C3H mouse with those of a Pb exposed woman in our control group. It may be seen that in Fig. 1, the levels of Se, Zn, Cu, Fe, and Cr in the hair of the mouse and the Pb-unexposed woman are practically identical. However, the hair Pb level was significantly higher, indicating that she was also, if less, Pb-exposed.

The 0.5 ppm Pb in the water lowered the level of Se in hair to about one half of the value of the unexposed mouse, demonstrating the effect of Pb on hair Se. This was a low exposure, but sufficient to abolish the anticancer effect of Se. As to the other elements, the 0.5 ppm Pb in the water had little or no effect on the levels of Zn, Cu, and Cr, but approximately doubled Fe and slightly lowered the Zn/Cu ratio. In our Pb-exposed subject, the hair Se concentration reached only one third of the value of the woman in Fig. 1. Her hair Pb were approximately eight times higher than the corresponding values of the unexposed human control. As Pb inhibits heme biosynthesis, her hair Fe values were increased to a similar extent. Furthermore, the exposure to Pb was also associated with a significant drop of the Zn/Cu ratio, to 1.1 from 11.2 in the unexposed control (see Fig. 2). All indications thus are that her exposure to Pb abolished the anticancer effect of Se and increased her breast cancer risk.

Conclusions

Evidence is presented here that the breast cancer patients of our study were chronically exposed primarily to Pb and that the level of this exposure was sufficient to abolish the cancer-protecting effects of Se. Effective measures to reduce the lead burden of the population thus must become an essential part of any public health program aiming at reducing the breast cancer risk of Nigerian women.

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