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Serum trace metals in diagnosis and prognosis of post-menopausal breast cancer in a tertiary health institution in Nigeria

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ABSTRACT

The levels of trace elements in the serum of postmenopausal breast cancer patients were determined and correlated with Tumour Parameters Index (TPI) ,developed for this study, in order to study their contributions in breast cancer diagnosis and evaluate their prognostic value. Serum zinc, selenium, lead, iron, copper, cadmium were estimated using atomic absorption spectrophotometry in 63 newly diagnosed postmenopausal breast cancer patients and compared with 63 apparently healthy controls. TPI was derived by a composite of six parameters that are conceptually relevant to tumour characteristics on a maximum 15-point scale and normality was assured. The results showed significantly higher mean levels of Pb(ug/L) (71.02±0.01)($p<0.0001$) and Cd(ug/L) (0.26± 0.03) in postmenopausal women with breast cancer than controls (52.03±0.02; 0.16±0.03) respectively ($p<0.03$). On the other hand, a significant decrease was observed in mean levels of Zn(ug/ml) (0.75±5.08 vs 1.04±3.19), Se(ug/L)(44.01±5.01 vs 69.37±4.6), Fe(ug/dl) (59.99 ±4.33 vs 82.94±1.71) and Cu (ug/dl) (84.51±2.39 vs 191.30±13.23) of postmenopausal women compared to the controls respectively. Multiple regression analysis showed that Pb and Cd were significantly positively correlated with TPI($\beta = 0.826$; $p < 0.0001$) and the R^2-values showed that 67.6% and 4.5% of variations observed in the outcome variable can be accountable to serum levels of the two trace elements respectively. Furthermore, Zn, Se, Fe and Cu were significantly inversely correlated with tumour parameters in such a way that 19.4%, 6.20%, 48.6% and 61.3% of variations observed in the outcome variable can be accountable to serum levels of Zn, Se, Fe and Cu respectively. Decrease in serum levels of Fe, Cu, Se, Zn and increase in levels of Pb and Cd may have potential value in predicting diagnosis and prognosis of breast cancer.

Key words: Breast Cancer, Postmenopausal women, Trace metals, Tumor Parameter Index.

INTRODUCTION

Breast cancer, whose absolute risk increases with age[1], is the most prevalent malignancy among women and a leading cause of cancer death in postmenopausal women[2] in both the developed and developing countries[3]. With a rise in incidence of breast cancer [4], about half the breast cancer cases and 60% of the deaths have been reported to occur in the economically developing countries [5]. In Nigeria, the incidence has so significantly risen that compared to historical records, a 100% increase has been reported during the last decade [6]. This observation calls for urgent attention to the control and management of cancer.

Much knowledge has accumulated on the aetiology and pathogenesis of this malignancy implicating genetic, hormonal and environmental factors. Trace elements are found naturally in the environment and human exposure

derives from a variety of sources, including air, drinking water and food. However, the levels of exposure to them are potentially modifiable [7].

Trace elements comprise metals in biological fluids at concentrations <1 μg/g of wet weight [8] and although they constitute a minor part of living tissues, it has become well established that many of these elements play essential role in a number of biochemical and physiological processes that confer cellular integrity and facilitate immune responses. Gowal et al., (2007) reported that a decline in the cell-mediated immunity predisposes a susceptible individual to cancer [9], and a close association has been found between immune responses and trace element status [10]. This could be through their action as activators or inhibitors of enzymatic reactions by competing with other elements and proteins for binding sites, by influencing the permeability of cell membranes, or through other mechanisms [11]. It is therefore reasonable to assume that these trace elements would exert action, directly or indirectly, on the carcinogenic process such as tumor growth, invasion and metastasis, especially when in abnormal expression [4]. While, copper, iron, selenium and zinc among others, are essential trace elements and as such are involved in maintaining health throughout life, lead and cadmium are non-essential and toxic with no known biological functions[8].

Some specific parameters are quite relevant to breast tumours. Some of these include; absence or presence of metastasis, tumour size, stage of tumour, tumour grade, number of breasts affected and number of palpable lymph nodes amongst others. These could be regarded as tumour parameters because, based on their outcome, certain deductions about the extent or severity of the breast cancer could be made. Deriving Tumour Parameter Index (TPI)(developed for this study), and considering its relationship with serum trace elements in these breast cancer patients, may be useful and significant and could therefore, serve as indicators of prognosis as the disease progresses.

Therefore, the aim of this study was to assess the levels of six trace elements ((zinc, (Zn), selenium (Se), lead (Pb), iron (Fe), copper (Cu), cadmium (Cd)) and correlate their levels with TPI. We hypothesized that TPI will be dependent on levels of trace elements and contribute significantly to tumour progression.

MATERIALS AND METHODS

PARTICIPANTS

Sixty three (63) newly diagnosed postmenopausal breast cancer patients, who did not have a first degree family history of cancer, were recruited from the surgical oncology clinics at the University College Hospital, Ibadan, Nigeria. The controls included age-matched 63 apparently healthy women recruited from members of staff of the University College, Ibadan, Nigeria. All study participants were asked to complete a self-administered questionnaire that included questions on demographic factors, medical history and health related behaviour. In order to ensure uniformity, all patients and controls were chosen from the same ethnic (Yoruba) background and living in the same southwestern part of Nigeria [as determined from their responses to questionnaires and clinical investigations]. The study was approved by the Joint University of Ibadan and University College Hospital Ethics Committee and an informed consent form was duly signed by each of the participant. Patients or controls with inflammations, women on regular haematinics or taking supplements, women with diabetes mellitus, hypertension, hepatitis, tuberculosis, jaundice, pregnancy or breast feeding subjects were excluded from this study.

Outcome Measures

Height

This was measured using a stadiometer and the readings were recorded in meter.

Weight

This was taken with Omron weighing scale (HBF,202) placed on a flat surface and the readings were recorded to the nearest 0.5 kg.

Body Mass Index (BMI)

This was calculated from the height and weight of the participants using the formula:

$$BMI\ (kg/m^2) = Weight\ (kg) / Height^2\ (m^2)$$

Collection of blood samples and measurement of trace element levels

Five ml of venous blood was obtained from anticubital fossa into plain sample bottle. The blood sample was allowed to clot and retract after which it was centrifuged in Centaur MSE centrifuge machine (Fisons, England) at 4000 rpm for 5 min to obtain serum which was stored at -20^{o}C until ready for assay.

Serum concentrations of copper, zinc, iron, lead, cadmium and selenium were determined by atomic absorption spectrophotometry (Buck 210VGP, USA).

Tumour Parameter Index (TPI)
In order to determine levels, and for purposes of evaluating measures in the study, rating scales were developed by coding specific parameters that are theoretically relevant to tumour characteristics in such a way that observations coded zero represented the lowest score possible indicating that the characteristic is absent. Tumour Parameter index (TPI) was measured as a composite scale on a maximum 15-point scale consisting of items regarding menopausal status- (pre menopause/post menopause), absence or presence of metastasis with response category of 0, 1 respectively; tumour size was defined as none(*Not present or visible*) and coded=0, very small(*when measured between 1.0 to 3.9mm*) and coded=1, small(*4.0 to 6.9 mm*) and coded=2, medium(*7.0 to 9.9 mm*) and coded=3 and large(*when measured between 10 mm and above*) and coded=4, while stage of tumour was characterized by histological characteristics of tumor staging of none=0, 1, 2, 3, 4. Breast affected was coded as none=0, only one=1 and both breasts=2 while number of lymph nodes palpable was coded as none=0, one =1, two=2, and more than two=3. Here high scores(>7) represented poor prognosis of cancer and indicated advancing pathogenesis while low scores(<7.0) represented better prognosis and early pathogenesis.

Statistical analysis
Data analysis was conducted using Statistical Package for Social Sciences (SPSS) version 21.

Descriptive statistics such as means, standard error of mean (SEM) and standard deviation (SD) were used to evaluate levels of parameters measured. Normality was assured for measures developed by aggregation of sub-variables. Independent t-Test statistic, ANOVA was employed to determine differences in mean scores between test groups and controls. Regression analysis was conducted to validate the association between dependent and factor variables in the study. The level of significance was set at $P = 0.05$ for all statistical procedures.

RESULTS

A total of 63 postmenopausal breast cancer patients and 63 apparently healthy age-matched controls were recruited. The mean age of the breast cancer patients was 52.97±1.49 years and that of controls was 54.33±1.63 years.

Table 1 compares the demographic and anthropometric measurement of breast cancer patients (subjects) with healthy controls.The mean BMI, percentage body fat (PBF), waist-hip-ratio (WHR), age at menarche, age at menopause, age at first pregnancy, parity and number of miscarriages/abortions were not significantly different from the controls.

Table 2 shows the mean levels of trace elements in cases and controls. There were statistically significant differences in the mean values of the trace elements. While the serum levels of Pb and Cd increased significantly, the serum levels of Zn, Se, Fe and Cu were significantly reduced.

Table 3 shows the regression between tumor parameter index (dependent variable) and trace elements (independent variable) in breast cancer patients.

The study measured mean TPI (9.871 ±2.06) for postmenopausal patients on a 15-point scale. Finally, subjecting the hypotheses that tumour parameter index is dependent on levels of trace elements among sample of participants to regression analysis and test of significance, resulted in the hypothesis being sustained.

Multiple regression analysis demonstrated significant positive correlation between Pb and TPI (Model Coefficient: $\beta = 0.826$; R^2 of 0.676 and $p < 0.0001$). The same pattern was observed between Cd and TPI (Model coefficient: $\beta = 0.254$; R^2 of 0.045 and $p < 0.0001$).

The same significant positive correlation between Cd and TPI was also observed in the multiple regression analysis (Model coefficient: $\beta = 0.254$; R^2 of 0.045 and $p < 0.0001$).

Furthermore, testing the variations in the outcome variable, TPI recorded suggested that 67.6% and 4.5% of variations observed in the outcome variable can be accountable to serum levels of Pb and Cd, the predictor variables respectively.

The Model coefficients computed from multiple regression analysis for Zn, Se, Fe and Cu were Zn ($\beta = -0.458$; R^2 of 0.194 and $p = 0.01$), Se($\beta = -0.284$; R^2 of 0.062 and $p = 0.045$),

Fe(β = -0.705; R^2 of 0.486 and $p < 0.0001$) and Cu(β = -0.788; R^2 of 0.613 and $p < 0.0001$), respectively.

These suggested that these trace elements Zn, Se, Fe and Cu were significantly inversely correlated with the outcome dependent variable in such a way that 19.4%, 6.20%, 48.6% and 61.3% of variations observed in the outcome variable can be accountable to serum levels of Zn, Se, Fe and Cu respectively, the predictor variable.

Table 1: Comparison of anthropometric indices in postmenopausal women with breast cancer and control

Parameter	SUBJECTS (N=63) Mean ± S.E	CONTROL (N=63) Mean ± SE	t-value	P-value
Age (years)	52.97±1.49	54.53±1.63	0.67	0.51
Systolic (mmHg)	132.67±3.43	139.63±3.36	1.37	0.18
Diastolic (mmHg)	86.10±2.36	85.11±2.17	-0.29	0.77
BMI (kg/m^2)	26.439±1.02	29.379±1.05	1.93	0.06
P.B.F (%)	26.416±1.51	28.927±1.48	2.02	0.07
W.H.R	0.88±0.02	0.80±0.04	-1.89	0.07
Cup size (cm)	36.48±0.66	36.95± 0.71	0.47	0.64
Age at menarche (yrs)	14.31± 0.33	14.30 ± 0.40	0.02	0.98
Age at menopause (yrs)	48.73± 1.13	49.67± 1.23	0.47	0.64
Age at first pregnancy (yrs)	26.50 ± 1.04	24.56± 1.20	1.18	0.24
Parity	3.67 ±0.41	3.87 ±0.43	0.31	0.75
Miscarriage/abortion	4.60± 1.75	2.20± 0.51	1.31	0.20

** = p<0.05 is significant*
BMI = body mass index
W.H.R = waist hip ratio
PBF= percentage body fat

Table 2: Mean (SE) of trace elements in breast cancer patients compared with healthy controls

Trace metals	SUBJECTS (N=63) Mean ± S.E	CONTROL (N=63) Mean ± SE	t-Values	P-Values
Selenium (ug/L)	44.01±5.01	69.37±4.60	2.199	0.033*****
Zinc (ug/ml)	0.75±5.08	1.0±3.19	4.082	0.000*****
Lead (ug/L)	71.02±0.01	52.03±0.02	11.84	0.000******
Iron (ug/dl)	59.99±4.33	82.94±1.71	6.967	0.000*****
Copper (ug/dl)	84.51±2.39	191.30±13.23	10.213	0.000*****
Cadmium (ug/L)	0.26±0.03	0.16±0.03	2.110	0.030*******

** = p<0.05 is significant*
***= p<0.0001 is significant*
**** = p<0.03 is significant*

Table 3: Regression table between tumour parameter index(dependent variable) and trace elements (independent variable) in breast cancer patients

Tumour parameter index (TPI)[φ]					
Trace elements	**R**	**R^2**	**β**	**t- value**	**p- value***
Zinc	0.458	0.194	-0.458	-3.574	0.010
Selenium	0.284	0.062	-0.284	-2.054	0.045
Lead	0.826	0.676	0.826	10.164	0.000
Iron	0.705	0.486	-0.705	-6.881	0.000
Copper	0.788	0.613	-0.788	-8.871	0.000
Cadmium	0.254	0.045	0.254	1.820	0.075

[φ] TPI: Mean Score of 9.871±2.06 on a 15-point scale of measure
** = p<0.05 is significant*

DISCUSSION

This study showed that anthropometric and demographic indices are similar in breast cancer patients and controls thus suggesting that lifestyles, diet and socio-cultural exposures are the same in the two groups of subjects.

Copper is involved in the cell metabolism, as a part of various enzymes tyrosinase, uricase and cytochrome oxidase, which are mainly concerned with oxidation reactions [12]. Angiogenesis, the growth of a tumor blood supply, is essential for tumor growth, invasion, and metastasis [2]. Molecular processes of angiogenesis require copper as an essential cofactor [13] and consistently high levels of copper have been found in breast cancer tissue [14]. In this study, serum levels of copper were significantly reduced in the breast cancer group when compared to the controls, thus supporting the uptake of Cu from the blood by cancer cells.

Increasing evidence has demonstrated that iron plays a significant role in the tumour microenvironment and pathways of iron acquisition, efflux, and regulation are all perturbed in cancer [15]. Iron deficiency has also been linked to increased angiogenesis. According to the report of Haung (2008), iron is an important cofactor for the enzyme, hypoxia inducible factor (HIF) - 1α prolyl-4-hydroxylase which hydroxylates HIF - 1α. Iron deficiency therefore, prevents hydroxylation of the proline and stabilizes the HIF- 1α protein which leads to increased angiogenesis [2]. The significant reduction in iron level in the breast cancer patients compared to the control could have increased the risk of development of breast cancer. This finding is in line with the report of Jian et al.,(2013), that Fe deficiency promotes human breast cancer growth and metastasis [15] . This is further buttressed by increased level of iron found in cancerous breast tissue of women in Egypt [14], although our work did not assess the level of iron in the breast tissues of the patients.

The primary gene protecting women from breast cancer, p53, has been reported to be the most frequently mutated or altered in the development of breast cancer [16]. This protein requires zinc as a cofactor, and if it is missing, the gene becomes mutated, resulting in it becoming inactivated or suppressed [17]. In our study, we observed a significant decrease in serum zinc level compared to the control. This observation in Zn level could be adduced to a mutated, inactivated or suppressed p53 gene resulting in uncontrolled cell division and oncogenic activation leading to development of breast cancer. This finding is in agreement with the report of Kacmarec et al. (2013), which observed reduced breast cancer risk in patients with high Zn level [18]. We also observed a significant negative association between Zn and tumor parameter index. The report of Silvena and Rohann, (2007), confirms this finding [19].

The mechanism of Se as an anticarcinogenic element is not well understood, but several speculative hypotheses have been made [20]. Se exerts its essential role in the formation and maintenance of the activity of glutathione peroxidase, (GSH-Px), a selenoenzyme that protects body against oxidative injury and free radical damage and consequently, mutation leading to neoplastic transformation of cells [21]. DNA mutation is a crucial step in carcinogenesis and increased number of oxidative DNA lesions have been noted in various tumours, strongly implicating such damage in the aetiology of cancer. The absence of selenium correlates with loss of GSH-Px activity and is associated with damage to cell membranes due to accumulation of free radicals [5]. In some studies, an inverse association between serum Se levels and neoplastic development has been observed in various cancer types [5]. It has also been postulated that the process underlying tumor development can lead to an uptake of Se by the malignant cells showing the human defense mechanisms against the neoplastic process [5]. The lower serum Se levels observed in our study could be attributed to either lower Se intake, or to sequestration of this element by the tumour cells or by both mechanisms. This finding is in agreement with the reports of Charalabopoulos et al., (2006)and Rejali et al. (2007) [22,23] .

Lead and Cd are non-essential toxic metals that have affinity for free sulphohydryl active site of enzymes and proteins. Reports have shown that Se interacts with Pb and Cd in vivo. These interactions are part of natural metal detoxification process, which result in the metabolic inactivation of Se. However, at sufficiently high exposure levels, Pb and Cd may overtime produce a state akin to Se deficiency thereby aborting the cancer-protecting effects of Se [4]. Our study returned a significant increase in the serum levels of Pb and Cd in breast cancer patients. These toxic trace metals have been reported to be involved in the generation of reactive oxygen species resulting in lipid peroxidation, DNA damage and altered gene expression [24]. The significant increase in the levels of Pb and Cd in the breast cancer patients observed in our study could have contributed to the pathogenesis of breast cancer.

The relationship and positive correlation observed between Pb and Cd with the TPI, suggests that the two trace metals would be risk factors rather than protective agents for breast cancer. Again, since the relationship and inverse correlation observed between TPI and Zn, Se, Fe and Cu, respectively, these trace metal could be seen as protective rather than risk factors. Other researchershave indeed found increased levels of Cu, Zn, Se and Fe in cancerous breast tissue[1,25,26], implying the reduction in serum level could have resulted from sequestration to the site of increased cellular activity. It has been suggested these elements compete for the binding sites in the cell, change its enzymatic activity and exert direct or indirect action on the carcinogenic process [27], thereby, accelerating the growth of tumours. Furthermore, the observed decrease in serum levels of Fe, Cu, Se, Zn and the increase in the levels of Pb and Cd may have prognostic potential in the management of breast cancer.

CONCLUSION

This study suggests that trace metals may be correlated with breast cancer risk. It also supports the hypothesis that TPI is significantly dependent on levels of serum trace elements and may significantly contribute to tumour progression. Although the exact mechanism responsible for the alterations in the levels of trace metals in these patients is unclear, the serum profile of these trace metals may have potential values in predicting prognosis of

breast cancer. This warrants further investigations on larger sample size, more other trace elements and multiple serum samples with different time intervals.

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