

Oestrogen levels and humoral immune parameters in Nigerian breast cancer patients

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Abstract

Objectives: Endocrine and immune interactions mediate breast cancer which is currently incurable. This study attempts at elucidating mechanisms by which breast cancer progresses by determining the levels of oestradiol and humoral immune parameters at different stages of breast cancer compared with women without breast cancer.

Patients and Methods: 59 subjects (29 breast cancer attending Surgery Clinics of 2 teaching hospitals in Lagos State, Nigeria and 30 controls) aged 23-82 years were recruited using convenience sampling. Serum was obtained from 10ml of fasting blood from each subject and stored frozen at -20°C until analysis. Oestradiol and albumin were estimated using commercial kits (Adaltis Italia S.P.A and Biolab SA, Maizy, France) respectively. Haptoglobin, alpha-2-macroglobulin, caeruloplasmin, IgG, IgA, IgM were analysed by single radial immuno-diffusion method. SPSS version 10 was used for statistical analysis.

Main Results: Oestradiol, haptoglobin and caeruloplasmin were significantly increased in premenopausal breast cancer patients compared with premenopausal controls, while only oestradiol was significantly increased in postmenopausal breast cancer patients compared with postmenopausal controls. Moreover, IgM, haptoglobin and caeruloplasmin were significantly reduced in stage III breast cancer patients compared with stages II and IV breast cancer patients.

Conclusions: Although inflammation is common to all stages of breast cancer, immunosuppression is most pronounced in breast cancer patients in stage III of the disease.

Key words: Breast cancer, immune response, acute phase proteins, immunoglobulins, Oestrogen.

Introduction

Breast cancer is the most frequently occurring malignant disease in women⁽¹⁾, and a significant health problem in industrialized western world⁽²⁾ and in developing countries⁽³⁾ including Ibadan, Nigeria⁽⁴⁾. Metastatic breast cancer is currently incurable because the exact aetiology is not entirely known, but it is considered as the outcome of a complex interplay amongst genetic, hormonal, immunological and environmental factors.^(5,6)

Oestrogens have a central role in the development and growth of hormone dependent breast tumours. However, similar concentrations of oestradiol in tumours with or without oestrogen receptors were observed by Purohit et. al⁽⁷⁾, suggesting that *in situ* synthesis rather than uptake from circulation might be considered the major pathway through which oestrogens originate. Osarogiagbon⁽⁸⁾ suggested that breast cancer might arise as a result of failure in immunologic surveillance at an age when the immunological function of the body is relatively inactive.

The interaction between oestrogen and the immune system through the production of cytokines was suggested as a possible mechanism by which breast cancer progresses.⁽⁹⁾ The emerging trend of cytokines being important regulators of oestrogen synthesis in breast cancer suggests an association between an increase in the risk of breast cancer and immuno-suppression.⁽⁸⁾ Increased

IL-6 level stimulates aromatase activity in breast tissue leading to excess production of oestrogen⁽¹⁰⁾. IL-6 is also a proinflammatory cytokine known to induce synthesis of acute phase proteins and immunoglobulin class switch to IgM. Therefore, it is likely that acute phase proteins are increased in breast cancer patients. This study attempts to find the relationship between oestradiol and humoral immune parameters in breast cancer patients. This may elucidate the mechanism by which breast cancer progresses and assist in the management of breast cancer patients.

Materials and methods

Convenience sampling method was used in this case-control study which was largely laboratory based after obtaining informed consent from the subjects and ethical clearance was also obtained from the Hospitals where the subjects were recruited.

Subjects

Fifty nine subjects aged 23-82 years were recruited for the study. Twenty nine were newly diagnosed breast cancer patients (14 premenopausal and 15 postmenopausal) recruited from the Surgery Clinics of the Lagos University Teaching Hospital (LUTH), Idiaraba, and the Lagos State University Teaching Hospital (LASUTH), Ikeja, both in Lagos metropolis, Nigeria. Nine (9), 16 and 4 of the cancer patients presented at stage II, III and IV respectively.

Thirty apparently healthy women (24 premenopausal and 6 postmenopausal) were recruited as controls. Patients or controls with inflammations, chronic diseases such as diabetes mellitus, hypertension, hepatitis, tuberculosis, jaundice, *Helicobacter pylori* infections or are pregnant, breastfeeding and on hormonal therapy were excluded from the study using standard laboratory methods.⁽¹¹⁾

Demographic/Anthropometric Indices

Demographic characteristics such as age at menarche, age at menopause, age at first pregnancy, parity and number of miscarriage/abortion were obtained from semi-structured questionnaire. Anthropometric indices such as height and weight were measured and body mass index (BMI) calculated as $\text{weight (kg)} / \text{Height}^2 (\text{m}^2)$ while waist and hip circumferences were measured and Waist to hip ratio calculated as waist circumference/ hip circumference.

Sample Collection

Ten ml of venous blood were obtained from the antecubital fossa vein after an overnight fast into plain sample bottles. The blood samples were allowed to clot and retract and then centrifuged in centaur MSE centrifuge machine (Fisons, England) at 500g for 5 minutes after which the serum was separated out and stored frozen at -20°C until ready for assay.

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Biochemical Analysis

Enzyme linked immunosorbent assay (ELISA) method was used for the estimation of Oestradiol using commercially manufactured kit (Adaltis Italia S.P.A). Albumin was estimated by BCG method described by Doumas and Biggs⁽¹²⁾ using a commercially manufactured kit (Biolabo SA, Maizy, France). Haptoglobin, alpha -2-macroglobulin, caeruloplasmin and Immunoglobulin classes (IgA, IgG and IgM) were analysed by the single radial immuno-diffusion method⁽¹³⁾.

Statistical Analysis

Statistical software-SPSS version 10 was used for analysis. Student's t-test was used to compare variables while Pearson correlation coefficient was used for closeness of association of demographic, anthropometric and biochemical indices. A $p < 0.05$ was regarded as statistically significant.

Results

Anthropometric and Demographic Characteristics

The mean age of the breast cancer patients was 47.17(2.68) years while that of controls was 40.07(2.24) years. Demographic characteristics such as age at menarche, age at menopause, age at first pregnancy, parity and number of miscarriage/abortion were not significantly different between breast cancer patients and controls ($p > 0.05$). Anthropometric indices (body mass index and waist to hip ratio) were also insignificantly different between breast cancer patients and controls (Table 1).

Table 1: Mean (SE) of Demographic and Anthropometric Measurements in Breast Cancer Patients and Healthy Controls

Index	Cases	Controls	T	P
Body mass index	27.36 (1.25) (n=29)	24.36 (1.09) n=(30)	1.80	0.08
Waist to hip ratio	1.38 (0.48) (n=29)	0.80 (1.31) (n=30)	1.21	0.23
Age at Menarche (yrs)	14.31 (0.33) (n=29)	14.30 (0.40) (n=30)	0.02	0.98
Age at Menopause (yrs)	48.73 (1.13) (n=15)	49.67 (1.23) (n=6)	0.47	0.64
Age at first pregnancy (ys)	26.50 (1.04) (n=26)	24.56 (1.20) (n=16)	1.18	0.24
Parity	3.67 (0.41) (n=27)	3.87 (0.43) (n=15)	0.31	0.75
Miscarriage/abortion	4.60 (1.75) (n=10)	2.20 (0.51) (n=10)	1.31	0.20

Oestradiol

Comparison of reproductive stages showed that oestrogen was significantly lower in postmenopausal than premenopausal breast cancer patients and controls ($p < 0.05$). Oestradiol level (Tables 3) was significantly higher in post menopausal breast cancer patients compared with postmenopausal healthy controls ($p < 0.05$).

Acute-phase proteins

Haptoglobin and caeruloplasmin were significantly elevated, while albumin was significantly reduced in breast cancer patients compared with the controls ($p < 0.05$) (Table 4). Albumin was significantly lower in

postmenopausal breast cancer patients compared with premenopausal breast cancer patients ($p < 0.05$) (Table 5). Haptoglobin and caeruloplasmin were significantly higher in premenopausal breast cancer cases, while albumin was significantly lower in premenopausal breast cancer cases compared with premenopausal patients ($p < 0.05$) (Table 3). Albumin was significantly reduced in postmenopausal breast cancer patients compared with their respective controls (Table 3). Haptoglobin and caeruloplasmin were significantly increasing while albumin level was significantly reducing from stage 2 to 4 of breast cancer patients (Table 7)

Table 2: Comparison of mean (SE) Oestradiol, Immunoglobulin Classes and Acute-Phase Proteins in Premenopausal Breast Cancer Patients and Healthy Controls

Index	Premenopausal Cases (n=14)	Premenopausal Controls (n=24)	T	p
Oestradiol (pg/mL)	200.00(22.95)	132.8(11.6)	2.90	0.06
IgG (g/L)	21.13(1.92)	24.5(1.98)	1.14	0.26
IgA (g/L)	3.00(0.73)	2.81(0.27)	0.29	0.77
IgM (g/L)	3.72(0.46)	2.73(0.27)	1.94	0.06
Haptoglobin (g/L)	1.25(0.13)	0.90(0.11)	1.99	0.05*
α_2 -Macroglobulin (g/L)	1.41(0.13)	1.32(0.13)	0.63	0.53
Caeruloplasmin (g/L)	0.51(4.37)	0.35(4.37)	3.53	0.00*
Albumin (g/L)	3.87(7.23)	4.25(8.56)	3.02	0.00*

Table 3: Comparison of the mean (SE) values of Oestradiol, Immunoglobulin Classes and Acute-Phase Proteins in Postmenopausal Breast Cancer Patients and Healthy Controls

Index	Postmenopausal Cases (n=15)	Postmenopausal Controls (n=6)	T	p
Oestradiol (pg/mL)	57.87(3.46)	37.50(3.11)	3.47	0.00*
IgG (g/L)	24.04(2.19)	28.64(6.34)	0.88	0.39
IgA (g/L)	5.39(0.95)	2.99(0.64)	1.51	0.15
IgM (g/L)	4.98(1.14)	3.49(0.36)	0.80	0.43
Haptoglobin (g/L)	1.82(0.27)	0.91(0.24)	1.94	0.07
α_2 -Macroglobulin (g/L)	1.49(0.14)	1.52(3.72)	0.13	0.89
Caeruloplasmin (g/L)	0.54(7.56)	0.45(5.91)	0.750	0.46
Albumin (g/L)	3.57(9.8)	4.16(0.12)	3.448	0.00*

Table 4. Comparison of mean (SE) of Immunoglobulin Classes and Acute-Phase Proteins in Breast Cancer Patients and Controls

Index	Cases (n=29)	Controls n=30)	T	p
IgG (g/L)	22.63 (1.47)	25.36(1.98)	1.09	0.28
IgA (g/L)	4.24(0.64)	2.84(2.47)	2.06	0.04*
IgM (g/L)	4.37(0.63)	2.89(0.24)	2.22	0.03*
Haptoglobin (g/L)	1.54(0.16)	0.90(9.81)	3.40	0.00*
α_2 -Macroglobulin (g/L)	1.45(9.87)	1.36(5.93)	0.79	0.43
Caeruloplasmin (g/L)	0.53(4.37)	0.37(2.15)	3.16	0.00*
Albumin (g/L)	3.72(6.67)	4.23(7.11)	5.02	0.00*

Immunoglobulins

IgA and IgM but not IgG were significantly elevated in breast cancer patients (Table 4) compared with the controls (< 0.05). No significant differences were observed in all immunoglobulin classes tested when postmenopausal was compared with premenopausal reproductive stage in breast cancer patients and controls ($p < 0.05$) (Table 5, 6). No significant differences ($p > 0.05$) were observed in IgG, IgM and IgA of premenopausal or postmenopausal breast cancer patients compared with their respective healthy controls (Table 2, 3). Comparison of the

mean levels of immunoglobulins at different stages of breast cancer showed that IgG, IgA and IgM were significantly reduced in stage 3 of breast cancer patients compared with stage 4. IgG appeared to be affected earlier (Stage 2) than IgA and IgM ($p < 0.05$, Table 7).

Correlation of Anthropometric, Demographic and Biochemical Indices

There were no significant correlations between anthropometric or demographic parameters and biochemical measurements (oestradiol, acute phase proteins and immunoglobulins) in cases and controls ($p > 0.05$). Oestradiol (Table 8) showed no correlation with immunoglobulin classes and acute-phase proteins in either the cases or controls ($p < 0.05$).

Table 5. Comparison of mean (SE) of Oestradiol, Immunoglobulin Classes and Acute-Phase Proteins in Premenopausal and Postmenopausal Breast Cancer Patients

Index	Premenopausal Cases (n=14)	Postmenopausal Cases (n=15)	T	P
Oestradiol (pg/mL)	200.00(22.95)	57.87(3.46)	6.33	0.00*
IgG (g/L)	21.13(1.92)	24.04(2.19)	0.99	0.33
IgA (g/L)	3.00(0.73)	5.39(0.96)	1.96	0.06
IgM (g/L)	3.72(0.46)	4.98(1.14)	0.99	0.33
Haptoglobin (g/L)	1.25(0.12)	1.82(0.27)	1.83	0.08
α_2 -Macroglobulin (g/L)	1.41(0.13)	1.49(0.14)	0.40	0.69
Caeruloplasmin (g/L)	0.51(4.37)	0.54(7.57)	0.37	0.71
Albumin (g/L)	3.87(7.23)	3.57(9.80)	2.39	0.02*

Table 6. Comparison of mean (SE) Oestradiol, Immunoglobulin classes and Acute-Phase Proteins in Premenopausal and Postmenopausal Healthy Controls

Index	Premenopausal Controls (n=24)	Postmenopausal Controls (n=6)	T	P
Oestradiol (pg/mL)	132.88(11.60)	37.50(3.11)	4.04	0.00*
IgG (g/L)	24.54(1.97)	28.64(6.34)	0.81	0.42
IgA (g/L)	2.81(0.27)	2.99(0.64)	0.29	0.77
IgM (g/L)	2.73(0.28)	3.49(0.36)	1.27	0.21
Haptoglobin (g/L)	0.90(0.11)	0.91(2.43)	0.034	0.97
α_2 -Macroglobulin (g/L)	1.32(7.16)	1.52(3.71)	1.38	0.18
Caeruloplasmin (g/L)	0.35(2.16)	0.45(5.91)	1.77	0.09
Albumin (g/L)	4.25(8.56)	4.16(0.12)	0.50	0.62

Table 7. Comparison of the mean (SE) values of Oestradiol, Immunoglobulin Classes and Acute-Phase Proteins in Different Stages of Breast Cancer Using Analysis of Variance (ANOVA)

Index	Stages of Disease			F	P
	(n=9) II	(n=16) III	(n=4) IV		
IgG (g/L)	21.18(2.85)	21.19(1.48)	31.67(4.59)	3.59	0.04*
IgA (g/L)	4.83(1.33)	3.25(0.67)	6.85(2.01)	2.11	0.14
IgM (g/L)	4.06(0.42)	3.38(0.31)	9.00(3.92)	6.10	0.01*
Haptoglobin (g/L)	1.07(0.18)	1.45(0.16)	2.99(0.42)	12.96	0.00*
α_2 -Macroglobulin (g/L)	1.54(0.22)	1.45(0.13)	1.26(0.13)	0.37	0.69
Caeruloplasmin (g/L)	0.42(4.37)	0.51(4.19)	0.83(0.21)	5.94	0.01*
Albumin (g/L)	3.93(8.74)	3.74(7.35)	3.14(3.65)	12.13	0.00*

Table 8. Correlation of Oestradiol with Acute-Phase Proteins and Immunoglobulin Classes in Cases and Controls

Index	Oestradiol r - , p - values	
	Cases (n=29)	Controls (n=30)
IgG	-0.14, 0.47	-0.17, 0.37
IgA	-0.22, 0.26	-0.10, 0.61
IgM	-0.12, 0.54	-0.19, 0.33
Haptoglobin	-0.35, 0.07	0.06, 0.74
α_2 -Macroglobulin	0.02, 0.92	-0.23, 0.22
Caeruloplasmin	-0.10, 0.60	-0.11, 0.57
Albumin	0.26, 0.17	-0.01, 0.96

Discussion

Epidemiological and experimental evidence implicates oestrogens in the aetiology of breast cancer.⁽¹⁴⁾ However, data on oestrogen levels in premenopausal women and breast cancer risk are sparse and conflicting⁽¹⁴⁾. The observed significant increase in oestradiol concentration in premenopausal breast cancer patients compared with postmenopausal breast cancer patients considered for the present study may be due to lack of ovarian production of oestradiol in post menopausal women.

Studies by Kanda and Tamaki⁽¹⁷⁾ demonstrated that oestradiol enhanced production of IgM and acute phase responses. Also, it was reported that IL-6 is an important regulator of oestrogen synthesis in breast cancer⁽⁹⁾. IL-6 (a Th 2 cell cytokine) induces hepatic synthesis of acute phase proteins as well as modulates immunoglobulin class switch.⁽¹⁰⁾ This may explain increases in IgM, IgA and acute-phase proteins (haptoglobin and caeruloplasmin) in breast cancer patients compared with controls as observed in this study.

It is possible that the progression of breast cancer patients is related to reproductive variables (menopause and oestrogen), stage of disease and immune responses. The significant elevation of haptoglobin ($p=0.00$), caeruloplasmin ($p=0.01$), IgG ($p=0.04$), and IgM ($p=0.01$) in stage IV of breast cancer compared with stage III suggests an immunosuppressive state and inflammatory responses in stage III of breast cancer. The implication of immuno-suppression observed in breast cancer patients, indicate a possible reason for their inability to fight infections resulting in mortality. Most (55%) of the patients in this study, were at stage III of the disease. At this stage, it is likely that there will be secondary bacterial infection which will lead to an acute phase responses as reported by the present study.

The increase in immunoglobulin classes in stage IV over stage III may be as a result of recovery from the immunosuppression. Therefore it is likely that only those that survive immunosuppressive stage III remained alive to develop stage IV breast cancer. In support of our observation, Vaso et al⁽¹⁸⁾ reported that strong humoral immune response could be induced in breast cancer patients by using synthetic peptides to improve immunity. Pike et al⁽¹⁹⁾ proposed that the effects of many established reproductive risk factors for breast cancer are mediated by hormonal mechanisms, especially oestrogens. This present study did not find any significant differences of established risk factors of breast cancer such as age at menarche, age at menopause, age at first pregnancy, parity

and number of miscarriage/abortion body mass index or waist to hip ratio in the patients and controls.

Oestradiol, haptoglobin and caeruloplasmin were significantly increased in premenopausal breast cancer patients compared with premenopausal controls, while only oestradiol was significantly increased in postmenopausal breast cancer patients compared with postmenopausal controls. Moreover, IgM, haptoglobin and caeruloplasmin were significantly reduced in stage III breast cancer patients compared with stages II and IV breast cancer patients. Based on these results, it may be postulated that different mechanisms probably exist in pre- and post-menopausal breast cancer patients; and that breast cancer patients are most immunosuppressed at the stage III of the disease.

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