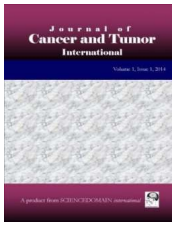

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Sex Hormones, Oestrogen Receptor, Progesterone Receptor and Human Epithelial Receptor 2 Expressions in Pre and Postmenopausal Sub-Saharan African Women with Breast Cancer

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Authors' contributions

This work was carried out in collaboration between all authors. Authors OOA, MACD, JIA and AFA designed the study, read and approved the final manuscript. Author OOA wrote the protocol and the first draft of the manuscript. Authors OOA and AFA enrolled the participants into the study. Authors OOA and MACD managed the literature search. Author OOA performed the laboratory and statistical analysis. Authors MACD, JIA and AFA critically reviewed the final manuscript. All authors read and approved the final manuscript.

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ABSTRACT

Aim: This study was carried out to determine the serum levels of sex hormones and gonadotropins as well as the expression of oestrogen receptor (ER), progesterone receptor (PR) and human epithelial receptor 2 (HER 2) in pre and postmenopausal women with breast cancer.
Study Design: Case-control study.
Place and Duration of Study: Surgical Oncology Clinic, University College Hospital, Ibadan, Nigeria between April 2011 and July 2014.
Methodology: 169 non-pregnant women aged 48.3±1.3 years were recruited for this study. They comprised of 85 histologically confirmed breast cancer pre-therapy (cases) matched with 84

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apparently healthy women without breast cancer (controls) according to age and menstrual phase. Both cases and controls were subdivided into pre and postmenopausal groups (54 premenopausal cases; 31 postmenopausal cases; 53 premenopausal controls; 31 postmenopausal controls respectively). Anthropometry and reproductive history were obtained by standard methods. Blood (10ml) was obtained from participants and centrifuged to obtain serum. Oestradiol, progesterone, follicle stimulating hormone (FSH) and luteinizing hormone (LH) were determined using enzyme immunoassay (EIA). Expression of ER, PR and HER 2 were determined by immunohistochemistry. Data analysed by Chi-square, Student's t-test and multiple regression were considered statistically significant at $p<0.05$.

Results: All premenopausal cases (100%) had ER and PR receptor negative expressions out of which 46(88.5%) had HER 2 receptor negative expression. Oestradiol and progesterone were significantly higher in postmenopausal cases compared with controls ($p<0.05$) while FSH and LH were significantly higher in premenopausal cases than controls ($p<0.05$).

Conclusion: The pathophysiology of breast cancer may be based on menstrual phase. Elevated gonadotropins and sex hormones may be important in premenopausal and postmenopausal women with breast cancer. Triple negative breast cancer observed in 88.5% of premenopausal women with breast cancer is critical in the management of the disease especially in younger women.

Keywords: Breast cancer; oestradiol; progesterone; gonadotropins; hormone receptors; enzyme immunoassay.

1. INTRODUCTION

Endogenous sex hormones have been reported to play a major role in the aetiology of breast cancer [1,2]. Premenopausal and postmenopausal women secrete sex hormones throughout their life time, though, with different regulatory pattern. The secretion of sex hormones is regulated mainly by the ovary in premenopausal women while, it is regulated by the adrenal glands in postmenopausal women [3]. 17β–oestradiol (E_2) is the most potent natural oestrogen secreted under the control of follicle stimulating hormone (FSH) and luteinizing hormone (LH). Follicle stimulating hormone stimulates the growth and recruitment of immature ovarian follicles in the ovary [4]. Additionally, it regulates aromatase activity, while LH is responsible for the actual production of androgens in the ovarian theca cells, thus providing the substrate for aromatization to oestrogens in the granulosa cells [5,3].

In breast cancer, the tumour grows within a hormonal milieu which has a decisive influence upon its development [6]. The role of oestrogens in breast cancer has been reported. It is thought that in promoting the growth of breast's end buds, oestrogens may contribute to an increase in cells that later in life become prone to cancerous growth [7]. The role of progesterone in breast cancer is controversial [8]. It has been hypothesised that its activity of opposing oestrogenic stimulation of the breast decreases breast cancer risk [9,8]. Contrarily, the risk of breast cancer is increased because breast mitotic rates are highest in the luteal phase of the menstrual cycle [10,9].

In spite of the several studies done on breast cancer,there is paucity of information on the association of sex hormones, gonadotropins and the expression of hormone receptors in Nigerian pre and postmenopausal women with breast cancer. This study therefore was designed to determine the association of sex hormones, gonadotropins with hormone receptors in Nigerian pre and postmenopausal women with breast cancer.

2. MATERIALS AND METHODS

The study was a prospective case-control study conducted in the Surgical Oncology Clinic of the Department of Surgery, University College Hospital, Ibadan, Nigeria. The study protocol was approved by the University of Ibadan and University College Hospital Health Review Committee. Informed consent was obtained from the participants before recruitment into the study. Participants were recruited between April, 2011 and March, 2014.

2.1 Study Participants

One hundred and sixty nine non pregnant 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 by the Surgical Oncologist from the Surgical Oncology Clinic of the Department of Surgery, University College Hospital, Ibadan. Eighty-four non-pregnant, apparently healthy women who served as controls were recruited at three Primary Health Clinics (PHC) in Ibadan North Local Government Area of Oyo State namely; 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. One of the controls was excluded from the study due to incomplete data on questionnaire and insufficient blood sample.

Each of the cases was matched for age and menstrual phases (follicular, luteal and postmenopausal) with the controls. Participants were reported as postmenopausal if they had stopped menstruating over the last twelve months [11]. Participants that had bilateral oophorectomy were also considered postmenopausal. Both cases and controls were subdivided into pre and postmenopausal groups (54 premenopausal cases; 31 postmenopausal cases; 53 premenopausal controls; 31 postmenopausal controls respectively).

2.2 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. Women with previous and recurrent breast cancer as well as postmenopausal women on hormone replacement therapy were also excluded.

2.3 Reproductive Indices

Data on reproductive history were obtained from semi-structured pre-test questionnaire administered to each participant.

2.4 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 [12].

2.5 Sample Collection

Ten millilitres of venous blood samples were drawn into plain bottle. For premenopausal participants, blood samples were drawn between days 5 and 9 of their menstrual cycle in follicular phase (forward dating) and 5 to 9 days before the anticipated start of their next menstrual cycle in the luteal phase (backward dating) [11]. The blood was allowed to retract and centrifuged at 3500 rpm for 5 minutes. The resulting serum was aliquoted and stored at -20℃ until analysis.

2.6 Hormonal Assay

Serum progesterone, E_2, LH and FSH were determined by Enzyme immune assay (EIA) on TOSOH AIA System Analyzers (Tosoh Corporation, Tokyo 105-8623, Japan).

2.7 Immunohistochemistry

Formalin fixed and paraffin embedded tissue samples from breast lesions of women with breast cancer were investigated by immunohistochemistry for the determination of oestrogen receptor (ER), progesterone receptor (PR) and human epithelial receptor 2 (HER 2).

2.8 Statistical Analysis

Data were analyzed using the statistical package for social scientists (SPSS 18.0) SPP, Inc., Richmond, CA. Chi-square test was used for categorical variables, Student's t-test was used for comparison of quantitative variables. Multiple regression analysis was employed to determine relationships between variables. $p<0.05$ was considered statistically significant.

3. RESULTS

Table 1 shows comparison of mean±S.E age, anthropometric, reproductive and hormonal indices among pre and postmenopausal women with breast cancer and their respective controls. Age was similar in pre and postmenopausal cases compared to their respective controls (***P***>.05). However, age of postmenenopausal cases and controls were significantly higher than premenopausal cases and controls respectively (***P***<.05).

All anthropometric indices in this study except BMI and all reproductive indices except number of previous miscarriages were significantly higher in premenopausal cases compared with controls (***P***<.05). Only weight and height were significantly higher while number of previous

miscarriages were lower in postmenopausal cases compared with controls (***P***<.05). No significant differences were observed in anthropometry or reproductive indices between postmenopausal cases and premenopausal cases (***P***>.05). However, body weight, waist circumference, hip circumference, waist hip ratio and waist height ratio were significantly higher in postmenopausal controls than premenopausal controls (***P***<.05) while no significant differences in reproductive indices were observed between these groups (***P***>.05).

The gonadotrophins-LH and FSH were significantly higher in premenopausal cases compared with premenopausal controls (***P***<.05). However, LH was significantly lower while E_2 and progesterone were higher in postmenopausal cases than controls (***P***<.05). LH and FSH were lower while E_2 and progesterone were higher in premenopausal than postmenopausal cases as well as premenopausal than postmenopausal controls (***P***<.05).

Table 2 shows the distribution of ER, PR and HER 2 positivity and negativity in the total number of women (cases) as well as pre and postmenopausal cases. High proportion of ER-, PR- and HER 2- expressions were observed in the three groups. In the cases, 69 (87.3%) were ER-, 71 (89.9%) were PR-while 64 (81.0%) were HER 2-. In premenopausal cases, 52 (100%) were ER-, 52 (100%) were PR- while 46(88.5%) were HER 2-. In postmenopausal cases, 17 (63.0%) were ER-, 18(66.7%) were PR- while17 (63.0%) were HER 2-. Although low, ER, PR and HER 2 positivity were also observed. 10 (12.7%) of the cases, 0 (0%) of the premenopausal cases and 10 (37.0%) of the postmenopausal cases had ER+ expression. 8 (10.1%) of the cases, 0 (0%) of the premenopausal cases and 9 (33.3%) of the postmenopausal cases had PR+ expression. 15 (19.0%) of the cases, 6 (11.5 %) of premenopausal cases and 10 (37.0%) of postmenopausal cases had HER+ expressions.

Table 1. Comparison of age, anthropometric measures, reproductive indices and hormones in pre and postmenopausal women with breast cancer and their respective controls

Variable	Prem cases n=54	Prem control n=53	Post cases n=31	Post control n=31	*P1*	*P2*	*P3*	*P4*
Age (years)	40.9±0.7	40.7±0.6	61.2±1.5	61.6±1.5	.85	.84	<.001*	<.001*
Anthropometric indices								
WC (cm)	88.5±1.4	78.3±1.3	92.2±1.7	89.8±1.5	<.001*	.34	.12	<.001*
HC (cm)	100.5±1.5	95.9±1.0	103.9±1.7	102.7±1.7	.01*	.62	.15	.001*
Wt (kg)	68.0±1.9	60.1±1.3	71.4±2.2	65.6±1.7	.001*	.01*	.26	.01*
Ht (m)	1.63±0.0	1.57±0.0	1.63±0.0	1.59±0.0	<.001*	.02*	.78	.08
BMI (kg/m^2)	25.7±0.7	24.5±0.5	26.8±0.7	25.7±0.7	.16	.22	.28	.11
WHR	0.88±0.0	0.81±0.0	0.89±0.0	0.88±0.0	<.001*	.48	.61	<.001*
WHtR	54.6±1.0	49.9±0.9	56.6±1.2	56.5±0.9	.001*	.93	.18	<.001*
Reproductive indices								
Age at Menarche	15.3±0.3	14.5±0.3	15.6±0.4	15.1±0.4	.04*	.40	.58	.58
NPP	4.8±0.3	2.6±0.3	5.4±0.5	6.5±0.4	<.001*	.09	.25	.25
NLB	3.3±0.3	0.2±0.2	3.9±0.5	4.7±0.3	.001*	.13	.27	.27
NIA	0.9±0.2	0.3±0.08	0.9±0.2	0.4±0.2	.002*	.06	.94	.94
Miscarriages	0.2±0.07	0.1±0.05	0.097±0.1	0.65±0.2	.68	.03*	.51	.51
Hormones								
Progest (nmol/L)	12.3±2.6	8.8±2.2	2.1±0.4	1.0±0.1	.31	.005*	.005*	.007*
E_2 (pmol/L)	452.8±43.3	430.8±46.5	156.5±12.4	90.4±3.6	.73	<.001*	<.001*	<.001*
LH (IU/L)	7.7±0.7	5.8±0.5	26.4±2.9	29.7±1.1	.02*	.29	<.001*	<.001*
FSH (IU/L)	7.2±0.6	5.6±0.4	60.9±6.4	79.6±4.1	.03*	.02*	<.001*	<.001*

values are mean±S.E, Prem cases=premenopausal women with breast cancer, Prem controls=premenopausal women without breast cancer, Post cases= postmenopausal women with breast cancer, post controls= postmenopausal women without breast cancer, WC=Waist circumference, HC=Hip circumference, Wt=Body weight, Ht=Height, BMI=Body mass index, WHR=Waist hip ratio, WHtR=Waist height ratio, NPP=Number of previous pregnancies, NLB=Number of live births, NIA=Number of induced abortion, Miscarriages=Previous miscarriages, Prog=Progesterone, E_2=Oestradiol, LH=Luteinizing hormone, FSH=Follicle stimulating hormone, p1=probability between Prem cases and Prem controls, p2=probability between post cases and post controls, p3=probability between premenopausal cases and postmenopausal cases, p4=probability between premenopausal controls and postmenopausal controls, Student's t-test was used for the comparisons between the groups

Table 2. Distribution pattern of hormone receptor positivity and negativity in tissue samples from women with breast cancer

Marker	Cases (%)	Pre cases (%)	Post cases (%)
Oestrogen receptor			
ER+	10 (12.7%)	0	10 (37.0%)
ER-	69 (87.3%)	52 (100%)	17 (63.0%)
Progesterone receptor			
PR+	8(10.1%)	0	9 (33.3%)
PR-	71 (89.9%)	52 (100%)	18 (66.7%)
HER 2			
HER 2+	15 (19.0%)	6 (11.5%)	10 (37.0%)
HER 2-	64 (81.0%)	46 (88.5%)	17 (63.0%)

ER=Oestrogen Receptor, PR= Progesterone receptor, HER 2=Human epithelial Receptor 2

Table 3 shows the expression pattern of the receptors in the total number of cases, premenopausal and postmenopausal women with breast cancer. Women with triple negative receptors accounted for 69.6% of the cases, 88.5% of premenopausal cases and 33.3% of the postmenopausal cases while triple positive receptors accounted for 2.5% of the cases and 7.4% of postmenopausal cases.

Table 3. Different expression patterns according to the positivity and negativity of ER, PR and HER2

n (%)	ER	PR	HER 2
55 (69.6%)	-	-	-
2 (2.5%)	+	+	+
2 (2.5%)	+	+	-
10 (12.7%)	-	-	+
5 (6.3%)	+	-	-
1 (1.3%)	+	-	+
2 (2.5%)	-	+	-
2 (2.5%)	-	+	+
Premenopausal (n=52)			
46 (88.5%)	-	-	-
6 (11.5%)	-	-	+
Postmenopausal (n=27)			
9 (33.3%)	-	-	-
2 (7.4%)	+	+	+
2 (7.4%)	+	+	-
4 (14.8%)	-	-	+
4 (14.8%)	+	-	-
2 (7.4%)	-	+	-
3 (11.1%)	-	+	+
1 (3.7%)	+	-	+

n=number of samples, ER=oestrogen receptor, PR= progesterone receptor, HER 2=human epithelial receptor 2

Table 4 shows the comparison of ER, PR and HER 2 between premenopausal and postmenopausal women with breast cancer. ER-, PR- and HER 2- were significantly higher in premenopausal women with breast cancer compared with postmenopausal women with breast cancer ($p<0.05$). There was no difference in cancer staging and site of affected breast ($p>0.05$).

Table 5 shows the multiple regression analysis of variables in pre and postmenopausal cases and controls. In premenopausal cases, progesterone had a significant and direct relationship with E_2 ($\beta=0.678$, $P<.05$) and vice versa ($\beta=0.575$, $P<.05$) while HER 2 had an inverse relationship with progesterone ($\beta=-0.245$, $P<.05$). In premenopausal controls, progesterone also had a positive relationship with E_2 ($\beta=0.413$, $P<.05$). In postmenopausal cases, PR had an inverse relationship while waist height ratio had a positive relationship with E_2 (($\beta=-0.450$, $\beta=7.311$ respectively, $P<.05$). Body mass index positively related with LH ($\beta=3.951$, $P<.05$). In postmenopausal controls, body mass index positively related with E_2 ($\beta=1.529$, $P<.05$) while body weight positively predicted LH ($\beta=1.888$, $P<.05$).

4. DISCUSSION

Follicle stimulating hormone controls E_2 level by negative feedback mechanism in premenopausal women [13]. High serum LH and FSH were reported to be associated with a significantly worse breast cancer prognosis in premenopausal breast cancer patients [14]. The ability of FSH to activate adenylyl cyclase thereby resulting in increased cAMP levels could be associated with its ability to induce breast cancer cell proliferation, differentiation and metastasis [15,16,4]. Comparison of E_2 level between premenopausal cases with controls showed no significant difference ($p>0.05$). Similar findings have been reported by others [17,8]. These findings implicate gonadotropin exposure in premenopausal breast carcinogenesis. Contrarily, serum FSH level was significantly lower while E_2 and progesterone levels were higher in postmenopausal cases compared with

controls (p<0.05) in this present study. Positive association of $E_2$with breast cancer risk in postmenopausal women has previously been observed [18]. Although, the reasons are not clear, low FSH level has also been observed in postmenopausal women with ovarian cancer [19,20]. However, breast and ovarian cancers are hormone-dependent cancers with genomic similarities [21]. Wang and co-workers observed high levels of progesterone in postmenopausal breast cancer [11]. Increased postmenopausal progesterone levels have also been implicated in dementia, with unknown reasons but may relate to small subclinical cerebral thrombosis [22,23]. Although, mechanisms involving FSH reduction and increased E2may underlie postmenopausal breast cancer in this study, it is uncertain if the elevated progesterone in the postmenopausal women in our study is related to menopause or breast cancer.

Table 4. Association of hormone receptor status, breast cancer stage and site of affected breast in pre and postmenopausal women with breast cancer

Variable	Premenopausal cases (n=52)	Postmenopausal cases (n=27)	χ2	*P*
Oestradiol receptor			22.050	<.001*
			Fishers	<.001*
ER+	0	10(37.0%)		
ER-	52(100%)	17(63.0%)		
Progesterone receptor			17.143	<.001*
			Fishers	<.001*
PR+	0	8(29.6%)		
PR-	52(100%)	19(70.4%)		
HER 2			5.488	.02*
			Fisher	.03*
HER 2+	6(11.5%)	9(33.3%)		
HER 2-	46(88.5%)	18(66.7%)		
Breast cancer stage	(n=54)	(n=31)	3.394	.34
			Fishers	
Stage 1	5(9.3%)	1(3.2%)		
Stage 2	3(5.6%)	5(16.1%)		
Stage 3	24(44.4%)	14(45.2%)		
Stage 4	22(40.7%)	11(35.5%)		
Breast Site	(n=54)	(n=31)	2.236	.14
Right Breast	30 (55.6%)	12(38.7%)		
Left Breast	24 (44.4%)	19(61.3%)		

*n=number of subjects, χ^2 =Chi-Squared test, Fishers=Fishers Exact ratio, p=probability, * = significant at p<0.05. HER2=Human epithelial receptor 2*

Table 5. Multiple regression analyses in pre and postmenopausal women with breast cancer and their respective controls

Groups	Dependent Variable	Predictors	β	t	*P*
Premenopausal cases					
R^2=0.532,F=8.920, ***P***<0.001	E_2	Progesterone	0.678	5.826	<.001*
R^2=0.574,F=10.548,***P***<0.001	Progesterone	E_2	0.575	5.263	<.001*
		HER 2	-0.245	-2.246	.03*
Premenopausal controls					
R^2=0.285,F=3.050,***P***=0.013	E_2	Progesterone	0.413	3.176	.003*
Postmenopausal cases					
R^2=0.392, F=3.545,***P***=0.022	E_2	PR	-0.450	-2.585	.02*
R^2=0.450, F=2.692, ***P***=0.034	E_2	WHtR	7.311	2.174	.04*
R^2=0.363,F=1.871,***P***=0.121	LH	Body mass index	3.951	2.095	.047*
Postmenopausal controls					
R^2=0.330,F=1.551,***P***=0.202	E_2	BMI	1.529	2.376	.03*
R^2=0.471, F=2.926,***P***=0.024	LH	body weight	1.888	2.893	.008*

*β=Beta coefficient, F=F statistics, t=Student's t-test, p=probability, *=significant, E_2= Oestradiol, LH= Luteinizing Hormone, FT_3=Triiodothyronine, TSH=Thyroid stimulating Hormone, ER=Oestrogen receptor, PR=Progesterone receptor, HER 2=Human epithelial receptor 2, WHtR=Waist Height Ratio. Multiple regression model was used for statistical analysis*

Visceral fat is more strongly associated with an adverse metabolic risk including insulin resistance and a strong aetiopathogenic factor for the development of type 2 diabetes mellitus [24,25]. Waist height ratio has been reported to correlate well with visceral obesity, higher values indicate higher risk of obesity-related cardiovascular disease [26]. These systemic effects could be involved in cancer biology [27,28].

In this present study, increased adiposity as indicated by higher levels of measures of waist circumference, hip circumference, body weight, height, waist hip ratio and waist height ratio in premenopausal women with breast cancer compared with controls ($p<0.05$). This is in tandem with a reported study [29]. Similarly, body weight and height were significantly higher in postmenopausal cases compared with controls ($p<0.05$). BMI, an indicator of general adiposity is positively related with E_2 in postmenopausal controls ($\beta=1.529$, $p<0.05$). Redistribution of body fat is peculiar to menopause [13]. The positive relationship between waist height ratio and E_2 in postmenopausal cases ($\beta=7.311$, $p<0.05$) suggest that visceral obesity may play a role in postmenopausal breast cancer implicating increased aromatase activity in postmenopausal women with breast cancer.

It would appear from these observations that increased adiposity may contribute to both pre and postmenopausal breast cancer development. However, visceral obesity also indicated by increased waist circumference was the most frequent component of metabolic syndrome in females in Nigeria. Increased adiposity associated with elevated E_2 was also observed in both pre and postmenopausal women with metabolic syndrome without breast cancer in Nigeria [13].

The underlying mechanisms of action of E_2 in the aetiology of breast cancer include the alkylation of cellular molecules, generation of active radicals and genotoxicity of oestrogen metabolites which are involved in initiation, promotion and progression of breast cancer [2,30]. Endocrine disruptors are known to accumulate in increased adipose tissue [12]. We therefore postulate that factors other than visceral obesity such as endocrine disruptors, may contribute to carcinogenesis.

Observations in this study showed that reproductive factors such as number of previous pregnancies, number of live births and number of induced abortions were significantly higher in premenopausal cases compared with premenopausal controls ($p<0.05$) Table 1. Induced abortion (IA) was reportedly significantly associated with an increased risk of breast cancer among Chinese females and the risk of breast cancer increased as the number of IA increased [31]. Age at menarche was also significantly higher in premenopausal cases compared with premenopausal controls ($p<0.05$). This is at variance with studies that found an association between early age at menarche and increased risk of breast cancer [32].

Triple negative breast cancers are poorly differentiated and are characterized by an aggressive clinical history. No specific treatment guidelines are currently available for this breast cancer sub-type. However, they are managed with standard treatment, which leaves them with a high rate of local and systemic relapse [33]. A large number of the cases, 55 (69.62%) were triple negative. Forty six (88.5%) cases were premenopausal while 9 (11.5%) were postmenopausal. Similar findings were reported in indigenous African women [34,35].

Oestrogens and progesterone function via binding to their corresponding intracellular receptors, ER and PR [36]. Oestrogen and progesterone receptors play important roles in the growth and differentiation of breast cancers. This makes them important prognostic markers [37,38,39]. The biologic, prognostic and predictive importance of assessment of ER expression in breast cancer is well established, the added value of PR assessment appears controversial in some climes [40,41,42,43,44]. The American Society of Clinical Oncology and the College of American Pathologists recommend testing for both ER and PR on all newly diagnosed cases of invasive breast cancer [45]. Studies show that the loss of PR expression was associated with worse prognosis among ER+ breast cancers [46,47,48,49,50]. Human epithelial receptor 2 also known as ErbB2-neu, located on chromosome 17q21 is also considered to be closely associated with the occurrence and development of breast cancer [51]. It is inactive under normal physiological conditions and upon activation; it may enhance tumour invasion and metastasis [52]. The knowledge of HER 2 status is important for treatment choice especially for patients with metastatic tumours who respond to Herceptin [53].

In this present study, oestrogen receptor negative (ER-) breast cancer was observed in 69 cases (87.34%), progesterone receptor negative (PR-) breast cancer was seen in 71 cases (89.87%) and HER 2 negative breast cancer was seen in 64 cases (81.01%). Similar observations were seen in both pre and postmenopausal cases. This suggests the predominance of hormone-receptor negative breast cancer in this study, corroborating the findings of Huo et al. [9]. A comparison of the expression of ER, PR and HER 2 between premenopausal and postmenopausal breast cancer participants in this study further showed the prominence of negative hormone receptors in premenopausal participants. Oestrogen receptor negative, PR negative and HER 2 negative expressions were significantly higher in premenopausal cases compared with postmenopausal cases ($p<0.001$, $p<0.001$ and $p=0.02$, respectively). The occurrence of negative hormone receptors in premenopausal cases could be a contributory factor to the aggressiveness of premenopausal breast cancer relative to postmenopausal breast cancer. There are reports that breast cancer in young women is diagnosed at more advanced stage with more aggressive tumour and are associated with higher mortality, shorter disease-free survival and more likely to recur after treatment both loco regionally and at distant sites than in older women [54-57].

In postmenopausal women with breast cancer, PR is inversely related to oestradiol ($\beta=-0.450$, $p=0.02$). The reason for this observation is not clear. Oestrogen receptor positive (ER+) was observed in 10 cases (12.66%). Progesterone receptor positive (PR+) and HER 2 positive were observed in 8 cases (10.13%) and 15 (18.99%), respectively. Similar findings in indigenous African women have been reported [8,52]. The observations in this study are at variance with the results obtained in blacks living in the United States and United Kingdom, in which higher proportion of the positive receptor expression were observed [58,59]. This observation might implicate geographic or environmental factors beyond genetics. Women with ER-positive breast cancer can benefit from endocrine therapy explaining their better survival outcomes [53]. Most evidence regarding the prognostic role of PR is based upon the assumption that PR expression indicates a functioning ER pathway [60]. Hence, PR-positive and ER-positive tumours have been reported to have a better response to endocrine therapy than ER-positive and PR-negative cancers [61].

5. CONCLUSION

Our findings implicate gonadotropin exposure in premenopausal breast carcinogenesis. Although, mechanisms involving FSH reduction and increased E_2 may underlie postmenopausal breast cancer in this study, it is uncertain if the elevated progesterone in the postmenopausal women in our study is related to menopause or breast cancer. Increased adiposity appears to contribute to the development of breast cancer in both pre and postmenopausal women. However, similar observations in apparently healthy women with metabolic syndrome in Nigeria, suggest that increased adiposity alone may not be a risk factor for breast cancer. The contribution of environmental factors to breast carcinogenesis may be important in Nigeria. We postulate that endocrine disruptors (environmental toxicants) may accumulate in increased adipose tissue and contribute to carcinogenesis. The predominance of triple negative receptor expression particularly in premenopausal women with breast cancer in Nigeria may explain the observed aggressiveness of the disease.

CONSENT

We declare that informed consent was obtained from all participants in the study after the details of the study was explained to them.

ETHICAL APPROVAL

We declare that the study protocol was examined and approved by the University of Ibadan/University College Hospital, Ibadan Ethics Committee and therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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