Hormonal Receptors, Human Epidermal Growth Factor Receptor-2 and Triple Negative Immunohistochemical Typing of Women Breast Cancer in Kampala, Uganda.

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Research Article

Keywords: Hormonal receptors, HER2, Triple negative, breast cancer, Uganda

Posted Date: June 22nd, 2020

DOI: https://doi.org/10.21203/rs.3.rs-36736/v1

Abstract

Background: The expression of estrogen and progesterone receptors and human epidermal growth factor receptor-2 has been reported to have invaluable prognostic role. This study aimed at determining the expression ER, PR and HER2 in women with breast cancer in Kampala, Uganda.

Methods: Expression of estrogen receptor (ER), progesterone receptor (PR) and human epidermal growth factor receptor-2 (HER2) was determined immunohistochemically. Logistic regression was performed to determine the effect of the independent factors in predicting the risk of not expressing the breast markers. A two-tailed $p<0.05$ was regarded to be statistically significant.

Results: ER, PR and HER2 were expressed in 53.4%, 46.6% and 18.5%, respectively. Co-expression of ER and PR and triple negative breast cancer was present in 42.7% and 37.9%, respectively. Age was an independent predictor of expression of ER (AOR = 0.18, 95% CI: 0.062-0.541, p = 0.002), PR (AOR = 0.35, 95% CI: 0.129-0.968, p = 0.043).

Conclusion: Majority of patients in this study had less than 50 years and the majority of them had infiltrating ductal carcinoma of no special type with grade 2. Age predicted independently the expression of both ER and PR in our study.

Background

Breast cancer (BC) is a leading cause of female cancer-related mortality globally particularly in those with more than 50 years and only 5 to 7% of patients in whom the disease is diagnosed before the fourth decade [1]. Worldwide the incidence of BC has been on the rise. According to International Agency for Research on Cancer (IARC) as reported by GLOBOCAN in 2012, it is estimated that, the global BC incidence is 11.9% ranking second after lung cancer [2].

In less developed areas, such as Africa and Middle East where population-based screening practice is still a challenge and yet the population on average is comprised of 20% of female patients who are diagnosed with BC before the age of 40 years [3, 4]. Nonetheless, the role of genetical predisposition or environmental factors in the pathogenesis of breast cancer for the premenopausal group is not clearly known as compared to those in the postmenopausal group [5].

The expression of hormonal receptors (ER and PR), HER2 protein and proliferation markers such as Ki67 in premenopausal women with BC differs from that in postmenopausal women [6–8]. In the study that was done in West Africa it was found that, hormonal receptors negative and triple negative breast cancer (TNBC) were representing the majority of cases [9]. Three studies done in Tanzania by Mbonde et al [10] reported 33% and 18% of ER and PR expression, respectively, Rambau et al [11] reported 32.7%, 42.3%, 23.1% and 38.4% for ER, PR, HER2 and TNBC, respectively and Mwakigonja et al [12] also reported 43.4%, 26.4%, 28%, 15.2% and 45.6% for ER, PR, HER2 and TNBC, respectively of the patients were expressing the markers and therefore, they were expected to benefit from hormonal therapy. In all the three studies,

most of the patients were of young age and had advanced stage at the time of presentation. Hormonal receptors and HER-2 protein expression together with other biological characteristics among women with breast cancer are generally comparable to those of other indigenous Africans and majority of patients have TN breast cancer [12].

Similar observation was reported in Kenya in a study which involved women with BC in whom ER and PR expression was reported to be 24% and 10%, respectively with advanced stage [13]. In Uganda studies have reported aggressive BC among women of young age and low expression of ER, PR and HER2. For example, Roy and Othieno reported that, women who are diagnosed with BC in Ugandan are of younger age and they tend to have aggressive tumour grade with low expression of the ER, PR, HER2 TNBC of 60%, 46%, 11% and 36%, respectively [14, 15]. In another study done in tertiary hospital in Uganda by Galukande et al [15] it reported 38%, 22% and 34% of the cases had ER, HER2 and TNBC, respectively.

This study aimed to determine the expression of hormonal receptors, HER2 and TNBC in women with BC in Uganda and the second objective was to determine the association of the expressed biomarkers with age, histological types and tumour grade.

Methods

Study design

This was analytical cross-sectional laboratory-based study which involved retrospective collection of the data.

Study setting

The study was conducted at the Department of Pathology, Makerere College of Health Sciences (MakCHS) in Kampala, Uganda. The department is situated at Mulago National Referral Hospital (MNRH). The department specifically serves the roles of teaching, research as well as offering diagnostic and autopsy services for the whole country.

Patients' specimens

The study utilized Formalin Fixed Paraffin Embedded (FFPE) tissue blocks of women who were diagnosed histologically with breast cancer from January 2010 to August 2017. The tissue blocks were retrieved from the laboratory archive and all the necessary clinical information of the patients were extracted from the laboratory request forms and supplementation of the clinical data was by means of the patients' files which were obtained from the medical record of the hospital.

Sampling Procedure

Purposive sampling method was employed for sampling the cases which were included in the study. All the sampled cases that met the inclusion criteria were retrieved consecutively until the sample size of 103 specimens of the FFPE tissue blocks was attained. The inclusion criteria were: (1) Female patients (2) Cases with available FFPE tissue blocks (3) Cases with available clinical information. The exclusion criteria were (1) Male patients (2) Missing FFPE tissue blocks (3) Cases with spoilt FFPE tissue blocks (4) Cases with missing clinical information.

H and E Staining

The sections were first placed on the cooling part of the embedding station at a temperature of -10 ^{0}C for 30 minutes before being sectioned. Thereafter, they were sectioned at thickness of 3 microns and placed on the slides and left in the oven at 50 ^{0}C for 30 minutes for dewaxing. The sections were furthermore dewaxed through three changes of xylene for 10 dips in each and then hydrated in grades of alcohol (100%, 95%, 90%, 75%, and 70%) followed by rinsing in running tape water.

This was followed by staining the tissue sections with Harris haematoxylin for 15 minutes. They were rinsed in running tap water for at least 5 minutes and then differentiated in 1% acid-alcohol (1% HCL in 70% alcohol) for 2 seconds. Sections were washed in running tap water and left to blue for 15 minutes then they were counter stained with eosin 1% for 5 minutes. They were washed in running tap water for 5 minutes. Then they were dehydrated in increased concentration of alcohol (70%, 90%, 95%, 100%) for a few seconds followed by washing in running tap water for 5 minutes. Finally, the slides were cleared in two changes of xylene for 10 dips and mounted with coverslips using Distyrene Plasticizer Xylene (DPX).

Reporting of the H and E

Reporting of the H and E stained tissue slides was done by two independent experienced pathologists who were blinded of the clinical presentation of the patients. Scoring of the tumour grade was done by using Bloom-Richard grading system and histological classification used in the present study was that of the WHO histological classification of breast cancer of 2010. Reporting of the tissue slides was done by two independent experienced pathologists who were blinded of the clinical information of the cases.

Immunohistochemistry (IHC) staining for hormonal receptors and HER2 protein antibodies

The deparaffinized tissue sections of 3 microns thickness were placed on charged glass slides (FrostStat, DAKO-Denmark) and then heated at 750 watts in a microwave for 10 minutes using 10 mmol/L of Tris Buffered Solution (TBS) of pH of 7.0. Sections were dipped in 3% of hydrogen peroxide solution for 10 minutes to block endogenous peroxidase activity in order to prevent background staining. Sections were rinsed in Phosphate Buffer Solution (PBS). Sections were pre-treated with secondary antibody amplifier

Horseradish Peroxidase (HRP) and rinsed in buffered water. The sections were then incubated with monoclonal mouse anti ER, PR and HER2 antibody (DAKO Company, Denmark) for 30 minutes at room temperature. Diaminobenzidene tetrahydrochloride (DAKO LSAB2, Denmark) solution was added onto the tissue sections for 10 seconds for detection purpose. Then the sections were counter stained with Harris haematoxyline for 30 seconds followed by making 10 dips of the slides in two changes of xylene, mounting the stained slides with DPX and lastly cover-slipping the slides.

Reporting of IHC stained tissue slides

This was carried out by two independent experienced pathologists apart from the ones who reported the histological tissue slides. The pathologists were blinded of both histological results and clinical information. Expression of ER and PR hormonal receptors was determined by using the guidelines provided in the American Society of Clinical Oncology/College of American Pathologists Guideline Recommendations for Immunohistochemical Testing of Estrogen and Progesterone Receptors in Breast Cancer as reported by Hammond et al [16]. In this study, scoring of ER/PR positive cases was considered if the percentage of positivity was not less than 1%. The percentage for the positivity was obtained by dividing the positively stained tumour cells over the total number of tumour cells and the fraction obtained was multiplied by 100 percent. Intensity of nuclear staining was ranging from 0–3 (0-negative, 1-weak, 2-moderate, 3-strong).

On the other hand, HER2 positivity was determined by HER2 test positive when IHC 3 + based on circumferential membrane staining that is complete and intense. HER2 test result as equivocal when IHC 2 + based on circumferential membrane staining that is incomplete and/or weak/moderate and within 10% of the invasive tumor cells or complete and circumferential membrane staining that is intense and within ≤ 10% of the invasive tumor cells. HER2 test result as negative when IHC 1 + as defined by incomplete membrane staining that is faint/barely perceptible and within 10% of the invasive tumor cells and IHC 0 as defined by no staining observed or membrane staining that is incomplete and is faint/barely perceptible and within ≤ 10% of the invasive tumor cells [16]. All IHC 2+, IHC 1 + and 0 test results in this study were considered negative.

Statistical analysis

Data collected were analyzed by using STATA version 13.0 (IBM Statistics, Chicago, USA). For checking errors and missing data were cross-checked by running frequency tables and crosstabs. Age of the patients was presented as mean ± standard deviation (SD). Prevalence of hormonal receptors (HRs) and HER2 expression were presented as proportion of all BC patients. Chi-Square statistical test was used to determine the statistical difference in expression of both HRs (ER and PR) and HER2 according to the clinicopathological characteristics (age, tumour grade and histological types). Logistic regression under univariate and multivariate analyses was used to model the prediction of the clinicopathological characteristics for the expression of ER, PR and HER2. Odds ratio (OR) at 95% confidence interval (CI)

was used to measure the risk of not expressing the HRs, expressing HER2 and being TN among the cases. A two tailed $p < 0.05$ was considered statistically significant.

Ethical consideration

We obtained ethical approval from the institution review board of the School of Biomedical Science of Makerere College of Sciences (MakCHS).

Results

Clinicopathological characteristics of the patients

A total of 103 patients were included in the present study. The mean ± SD age of the patients was 49 ± 15 years (range: 24–93 years). Thirty patients (29.1%) were in age group 41–50 years, while 35.0% (36/103) were below 41 years. Regarding the histological types of breast cancer, majority 68.9% (71/103) of the patients had invasive carcinoma of no special type (NST) and 22.3% (23/103) had invasive lobular carcinoma. The histological grading indicated that, more than half 53.4% (55/103) of the patients had intermediate grade, and 31.1% (32/103) had high tumour grade (Table 1).

Table 1
Clinicopathologic characteristics of the patients

Variable	Frequency N = 103	Percentage (%)
Age (Years)		
≤41	36	35.0
41–50	30	29.1
> 50	37	35.9
Histological type		
Invasive carcinoma of not specified type (NST)	71	68.9
Invasive lobular carcinoma	23	22.3
Invasive micropapillary carcinoma	4	3.9
Mucinous carcinoma	3	2.9
Tubular carcinoma	1	1.0
Ductal carcinoma in situ	1	1.0
Bloom-Richardson grading system		
Grade 1	16	15.5
Grade 2	55	53.4
Grade 3	32	31.1

High grade cancer was highest 16/32 (50.0%) among those aged 41 years and below, and least 5/32 (15.6%) among those aged above 50 years (Fig. 1).

Expression of ER, PR and co-expression of ER and PR

ER was the most expressed HR and more than half of the patients 53.4% (55/103) had positive ER (Fig. 2a) followed by PR that was positive in 46.6% (48/103) of the patients (Fig. 2b). Co-expression of ER and PR was found in 42.7% (44/103). For all ER, PR and co-expression of ER and PR, the level of expression of the hormonal receptors was increasing with increase in the age of the patients. This means, the level of expression of the hormonal receptors was lower in young patients than in old patient. The difference in expression of ER (95% CI: 0.081–0.594, p = 0.007), PR (95% CI: 0.070–0.525, p = 0.003*)* and co-expression of ER and PR (95% CI: 0.047–0.394, p = 0.000) was statistically significant. The expression of ER, PR and co-expression of ER and PR in this study according to tumour grades and histological types of breast cancer among the cases was not statistically significantly different (Table 2).

Table 2
Expression of ER, PR, and co-expression of ER and PR according to age, histological type and tumour grade

Age (Years)	ER+ (n = 55)	ER- (n = 48)	PR+ (n = 48)	PR- (n = 55)	ER+ & PR+ (n = 44)	ER- & PR- (n = 59)
41	13(23.6)	22(45.8)	10(20.8)	25(45.5)	7(15.9)	28(47.5)
41–50	15(27.3)	16(33.3)	13(27.1)	18(32.7)	13(29.5)	18(30.5)
> 50	27(49.1)	10(20.8)	25(52.1)	12(21.8)	24(54.5)	13(22.0)
P-value	*0.007*		*0.003*		*0.000*	
95% CI	*0.081–0.594*		*0.070–0.525*		*0.047–0.394*	
Histological type						
IDC-NST	38(69.1)	33(68.8)	31(64.6)	40(72.7)	30(68.2)	41(69.5)
ILC	11(20.0)	12(25.0)	13(27.1)	10(18.2)	10(22.7)	13(22.0)
Others	6(10.9)	3(6.3)	4(8.3)	5(9.1)	4(9.1)	5(8.5)
P-value	*0.630*		*0.643*		*0.773*	
95% CI	*0.528–2.812*		*0.356–1.893*		*0.486–2.643*	
B-R grading						
Grade 1	9(16.4)	7(14.6)	8(16.7)	8(14.5)	8(18.2)	8(13.6)
Grade 2	29(52.7)	26(54.2)	28(58.3)	27(49.1)	25(56.8)	30(50.8)
Grade 3	17(30.9)	15(31.3)	12(25.0)	20(36.4)	11(25.0)	21(35.6)
P-value	*0.969*		*0.461*		*0.490*	
95% CI	*0.10-1.0*		*0.491–1.510*		*0.473–1.493*	

ER- Estrogen receptor, PR-Progesterone receptor, B-R-Bloom-Richardson grading system, IDC-NST-Infiltrating ductal carcinoma-Not specified type, ILC-Invasive lobular carcinoma, CI-Confidence interval, P-P-value

Expression of HER2 protein and triple negative breast cancer (TNBC) in the study

Expression of HER2 (Fig. 2c) in this study was present in 18.5% (19/103) whereas triple negative breast cancer (TNBC) was present in 37.9% (39/103) of all the patients.

HER2 expression and TNBC cases were predominantly found in premenopausal patients as compared to the postmenopausal patients. The difference in expression for HER2 in the study cases was statistically significantly different (95% CI: 1.011–2.109, p = 0.04) but for TNBC was not statistically different (95% CI: 0.789–6.331, p = 0.09). The expression of HER2 and being triple negative for the cases included in this study according to tumour grades and histological types of breast cancer among the cases was not statistically significantly different (Table 3).

Table 3
Expression of HER2 and TNBC according to age, histological type and tumour grade.

Age (Years)	HER2+ (n = 19)	HER2- (n = 84)	TNBC (n = 39)	Not TNBC (n = 64)
< 41	3(15.8)	32(38.1)	17(43.6)	18(28.1)
41–50	10(52.6)	21(25.0)	13(33.3)	18(28.1)
>50	6(31.6)	31(36.9)	9(23.1)	28(43.8)
P value	*0.042*		*0.090*	
95% CI	*1.011–2.109*		*0.789–6.331*	
Histological type				
IDC-NST	12(63.2)	59(70.2)	29(74.4)	42(65.6)
ILC	7(36.8)	16(19.0)	7(17.9)	16(25.0)
Others	0(0.0)	9(10.7)	3(7.7)	6(9.4)
P value	*0.114*		*0.624*	
95% CI	*0.332–2.838*		*0.520–2.970*	
B-R grading				
1	1(5.3)	15(17.9)	7(17.9)	9(14.1)
2	10(52.6)	45(53.6)	22(56.4)	33(51.6)
3	8(42.1)	24(28.6)	10(25.6)	22(34.4)
P value	*0.287*		*0.626*	
95% CI	*0.319–2.338*		*0.631–4.649*	

HER-2- Human epidermal receptor-2, TNBC-Triple negative breast cancer, B-R-Bloom-Richardson grading system, IDC-NST-Infiltrating ductal carcinoma-Not specified type, ILC-Invasive lobular carcinoma, CI-Confidence interval, P-P-value

Prediction of clinicopathologic characteristics for the expression of hormonal receptors and HER2 protein

The expression of ER in pre-and pos-menopausal women with BC in this study was almost equal. Univariately, post-menopausal women (> 50 years) were 3.7 times more likely to express ER compared to pre-menopausal women (≤ 50 years) (72.92% versus 42.42%) and the difference for expressing ER between the two groups compared was statistically significantly different (95% CI: 1.53–8.79, p = 0.004). When factors were adjusted for each other, age remained an independent predictor of expression of ER and post-menopausal women had 72% chances more likely to express ER than pre-menopausal women and the difference remained statistically significantly different (95% CI: 0.062–0.541, p = 0.002) (Table 3). In our study it was found that, cases with grade 2 were 7% less likely to express ER compared to cases with those who had grade 1 whereas those with tumour grade 3 were 22% less likely to express ER compared to cases with grade 1. However, in both compared groups the difference was not statistically significant (Table 3). Cases with invasive lobular carcinoma (ILC) were 42% more likely not to express ER than those with infiltrating ductal carcinoma of no specified type (IDC-NST). Other types of breast cancer were 54% more likely not to express ER than those with IDC-NST. The risk of not expressing the ER for ILC and other types of breast cancer as compared to IDC-NST in our series was not significantly different.

Table 3
Univariate and multivariate analyses of the association between ER expression and age, tumour grade and histological types of breast cancer in the patients

	ER expression					
Variable	**Yes (n = 55)**	**No (n = 48)**	**COR (95% CI**	**P**	**AOR (95% CI**	**P**
Age group (Years)						
≤ 50	28(50.9)	38(79.2)	1.0			
> 50	27(47.1)	10(20.8)	3.66(1.53–8.79)	0.004	0.18(0.062–0.541)	0.002
B-R grading						
Grade 1	9(16.4)	7(14.6)	1.0			
Grade 2	30(54.6)	25(52.1)	0.93(0.30–2.86)	0.904	-	-
Grade 3	16(29.1)	16(33.3)	0.78(0.23–2.60)	0.680	-	-
Histological type						
IDC-NST	38(69.1)	33(68.8)	1.0			
ILC	11(20.0)	12(25.0)	0.58(0.133–2.485)	0.459	-	-
Others	6(10.9)	3(6.3)	0.46(0.092–2.292)	0.342	-	-

ER- Estrogen receptor, COR-Crude odds ratio, AOR-Adjusted odds ratio, B-R-Bloom-Richardson grading system, IDC-NST-Infiltrating ductal carcinoma-Not specified type, ILC-Invasive lobular carcinoma, P-P-value

Association of patients' clinicopathologic characteristics with PR expression

Under univariate analysis, post-menopausal women were 3.9 more likely to express PR hormonal receptor than pre-menopausal women (67.6% versus 34.8%) and the difference was statistically significantly different (95% CI: 1.66–9.15, p = 0.002). Even in multivariate analysis, age remained a predicting factor for not expressing PR hormonal receptor in this study. Post-menopausal women were 65% more likely not to express the PR hormonal receptor and the difference was statistically significant (95% CI: 0.129–0.968) (Table 4). There was almost no difference in the risk of not expressing PR between cases with grade 2 and those with grade 1 (COR = 1.04, 95% CI: 0.344–3.316, p = 0.949) whereas those with tumour grade 3 were 40% less likely to express PR compared to cases with grade 1. However, in both compared

groups the difference was not statistically significant (Table 4). Cases with invasive lobular carcinoma (ILC) were 3% more likely not to express ER than those with infiltrating ductal carcinoma of no specified type (IDC-NST). Other types of breast cancer were 1.63 more likely not to express ER than those with IDC-NST, however, there was no statistical difference (Table 4).

Table 4
Univariate and multivariate analyses for the association between PR expression and age, tumour grade and histological types of breast cancer in the patients

	PR expression					
Variable	**Yes (n = 48)**	**No (n = 55)**	**COR (95% CI)**	**P**	**AOR (95% CI)**	**P**
Age group (Years)						
≤ 50	23(47.9)	43(78.2)	1.0		0.1	
> 50	25(52.1)	12(21.8)	3.89(1.66–9.15)	0.002	0.35(0.129–0.968)	0.043
B-R grading						
Grade 1	8(16.7)	8(14.6)	1.0			
Grade 2	28(58.3)	27(49.1)	1.04(0.344–3.316)	0.949	-	-
Grade 3	12(25.0)	20(36.4)	0.60(0.18–2.02)	0.409	-	-
Histological type						
IDC-NST	31(64.6)	40(72.7)	1.0			
ILC	13(27.1)	10(18.2)	0.97(0.240–3.913)	0.964	-	-
Others	4(8.3)	5(9.1)	1.63(0.344–7.670	0.540	-	-

PR- Progesterone receptor, COR-Crude odds ratio, AOR-Adjusted odds ratio, B-R-Bloom-Richardson grading system, IDC-NST-Infiltrating ductal carcinoma-Not specified type, ILC- Invasive lobular carcinoma, P-P-value

Association of patients' clinicopathologic characteristics with co-expression of ER and PR hormonal receptors

Post-menopausal women were 3.86 times more likely to have co-express of ER and PR hormonal receptors than pre-menopausal women in this study (88.9% versus 30.3%) and the difference between the two compared groups was significantly different (95% CI: 1.659–8.968, p = 0.001). Under multivariate

analysis, age became not an independent predictor of co-expression of ER and PR hormonal receptors although post-menopausal women had 14% more chance of having co-expression of ER and PR than pre-menopausal women but there was no statistical difference (95% CI: 0.142–1.051, p = 0.063) (Table 5). Grade 2 and 3 cases were 1.91 and 1.59 more likely to have co-expression of ER and PR, respectively but there was no statistical significantly different when both were compared to cases with tumour grade 1 (95% CI: 0.563–6.477, p = 0.300) and (95% CI: 0.645.3.921, p = 0.313. Cases with invasive lobular carcinoma (ILC) were 8% less likely not to have co-express of ER and PR than those with IDC-NST and other types of breast cancer were 4% less likely not to express ER than those with IDC-NST, however, there was no statistical difference (Table 5).

Table 5
Univariate and multivariate analyses for the association of co-expression of ER and PR and age, tumour grade and histological types of breast cancer in the patients

	ER and PR co-expression					
Variable	**Yes (n = 44)**	**No (n = 59)**	**COR (95% CI)**	**P**	**AOR (95% CI)**	**P**
Age group (Years)						
≤ 50	20(45.5)	45(76.3)	1.0		1.0	
> 50	24(54.5)	14(23.7)	3.86(1.659–8.968)	0.001	0.86(0.142–1.051)	0.063
B-R grading						
Grade 1	8(18.2)	8(13.6)	1.0			
Grade 2	25(56.8)	30(50.8)	1.91(0.563–6.477)	0.300	-	-
Grade 3	11(25.0)	21(35.6)	1.59(0.645.3.921)	0.313	-	-
Histological type						
IDC-NST	30(68.2)	41(69.5)	1.0			
ILC	10(22.7)	13(22.0)	0.92(0.226–3.696)	0.900	-	-
Others	4(9.1)	5(8.5)	0.96(0.204–4.539)	0.960	-	-

ER & PR- Estrogen receptor and progesterone receptor, COR-Crude odds ratio, AOR-Adjusted odds ratio, B-R-Bloom-Richardson grading system, IDC-NST-Infiltrating ductal carcinoma-Not specified type, ILC-Invasive lobular carcinoma, P-P-value

Association of patients' clinicopathologic characteristics with HER2 expression

There was no statistically significant association between patients' age and HER2 expression even for univariate analysis (95% CI: 0.27–2.29, p = 0.663) but post-menopausal women (> 50 years) had 21% less chance of expressing HER2 protein compared to pre-menopausal women (≤ 50 years) (31.6% versus 68.4%) (Table 6). HER2 expression which is an indication of poor prognosis was increasing with increase in the tumour grades. Cases with tumour grade 2 and 3 were 3.33 and 5 times more likely to have positive HER2, respectively but the difference for both grades with those having tumour grade 1 was not significantly different (95% CI: 0.39–28.25, p = 0.27) and (95% CI: 0.57–44.08, p = 0.147). The histological types of BC among the cases could not predict the risk of not expressing PR in univariate analysis.

Table 6
Univariate and multivariate analyses for the association between HER2 expression and age, tumour grade and histological types of breast cancer in the patients.

	HER2 expression					
Variable	**Yes (n = 19)**	**No (n = 84)**	**COR (95% CI**	**P**	**AOR (95% CI**	**P**
Age group (Years)						
≤ 50	13(68.4)	53(63.1)	1.0			
> 50	6(31.6)	31(36.9)	0.79(0.27–2.29)	0.663	-	-
B-R grading						
Grade 1	1(5.3)	15(17.9)	1.0			
Grade 2	10(52.6)	45(53.6)	3.33(0.39–28.25)	0.27	0.10(0.010–1.048)	0.055
Grade 3	8(42.1)	24(28.6)	5.0(0.57–44.08)	0.147	0.48(0.141–1.613)	0.233
Histological type						
IDC-NST	12(63.2)	59(70.2)	1.0			
ILC	7(36.8)	16(19.0)	0.72(0.54–9.02)	0.116	0.33(0.401–11.63)	0.713
Others	0(0.0)	9(10.7)	1.03(0.35–3.01)	0.957	-	-

HER2-Human-epidermal receptor-2, COR-Crude odds ratio, AOR-Adjusted odds ratio, B-R-Bloom-Richardson grading system, IDC-NST-Infiltrating ductal carcinoma-Not specified type, ILC_Invasive lobular carcinoma, P-P-value

Association of patients' clinicopathologic characteristics with triple negative expression

The risk of post-menopausal women (> 50 years) to be triple negative was 61% less than pre-menopausal women (≤ 50 years) (23.1% versus 76.9%) and the difference was statistically significantly different (95% CI: 0.16–0.94, p = 0.037) for univariate analysis. Also age continued to be an independent predicting factor under multivariate analysis of being triple negative between pre-and pos-menopausal cases. Women with breast cancer aged not more than 50 years were 4.4 times more likely to be triple negative than those aged more than 50 years and the association was significantly different (95% CI: 1.451–13.223, p = 0.009). Tumour grade was increasing with increase in the proportion of triple negative cases.

Cases with tumour grade 2 and 3 had 21% and 33% more likely to be triple negative than grade 1, respectively however, the difference was not statistically significantly different (95% CI: 0.26–2.45, p = 0.69) and (95% CI: 0.20–2.30, p = 0.53), respectively. Cases with ILC were 60% less likely to be triple negative whereas those with other histological types were 20% less likely to be triple negative. However, the difference in the chances of being triple negative for the two compared histological types was not statistically significant (95% CI: 0.21–3.51, p = 0.31) and (95% CI: 0.34–1.92, p = 0.62).

Table 7
Univariate and multivariate analyses for the association between TNBC and age, tumour grade and histological types of breast cancer in the patients

	TNBC expression					
Variable	**Yes (n = 39)**	**No (n = 64)**	**COR (95% CI)**	**P**	**AOR (95% CI)**	**P**
Age group (Years)						
≤ 50	30(76.9)	36(56.3)	1.0			
> 50	9(23.1)	28(43.8)	0.39(0.16–0.94)	0.037	4.38(1.451–13.223)	0.009
B-R grade						
Grade 1	7(18.0)	9(14.1)	1.0			
Grade 2	21(53.9)	34(53.1)	0.79(0.26–2.45)	0.69	-	-
Grade 3	11(28.2)	21(32.8)	0.67(0.20–2.30)	0.53	-	-
Histological type						
IDC-NST	29(74.4)	42(65.6)	1.0			
ILC	7(19.9)	16(25.0)	0.4(0.21–3.51)	0.31	-	
Others	3(7.7)	6(9.4)	0.8(0.34–1.92)	0.62	-	-

TNBC-Triple negative breast cancer, COR-Crude odds ratio, AOR-Adjusted odds ratio, B-R-Bloom-Richardson grading system, IDC-NST-Infiltrating ductal carcinoma-Not specified type, ILC- Invasive lobular carcinoma, P-P-value

Discussion

Hormonal receptors (HRs) and HER2 characterization of patients with BC has been widely documented in the literature with entire focus on prediction and prognostic roles of the biomarkers when they are examined in conjunction with the conventional clinicopathological prognostic factors for BC. Although a

number of studies agree on the positive correlation of such biomarkers with age, tumour grade among many other conventional prognostic factors, still there is quite a significant number of studies in the literature reporting contradicting results.

We determined the prevalence of expression of ER, PR, co-expression of ER and PR, HER2 and in whom the three breast markers were all not expressed (TNBC). Regarding the important findings for this study is that, HRs expression was higher in postmenopausal than in premenopausal women. HER2 protein expression was higher in premenopausal patients compared to postmenopausal ones. Likewise, there were more patients aged not more than 50 years with TNBC than patients who had more than 50 years. Interestingly, this study found that, age was a powerful independent predictor for the positive association of with ER, PR and TNBC.

Majority of patients with BC among blacks and even those from developing countries have been reported to be of young age unlike in Caucasians and other developed countries in which patients with BC tend to be aged more than 50 years. Majority of the patients in our study 64.1% (66/103) were aged not more than 50 years (Table 1) with mean ± SD age 49 ± 15 years. This finding is in agreement with other studies that were conducted in Tanzania (mean = 48.3 years and 49.0 years), Uganda (mean = 45.0 years), Ghana (mean = 51.0 years) and Egypt (mean = 51.3 years) [11, 12, 15, 17, 18]. The mean age of patients with BC reported in most developing countries is lower than that reported in developing countries. Adjei et al reported a mean age of 60.2 years in Norwegian women with BC [17]. Additionally, the varsity majority of patients with BC in developing countries particularly who are living in the southern part of the Sub-Saharan region are 50 years or younger [15]. In our study 64.1% of the patients had 50 years or younger similar and even slightly lower than two studies which were previously done in Uganda which reported that, 68.8% and in another study 73% of the patients had 50 years or younger [14].

Furthermore, younger patients aged 50 years or younger in our series had high tumour grade (grade 3) as compared to those aged above 50 years (26.2% versus 4.9%) (Fig. 1). This is similar to the reports in most of the African countries that reported aggressive BC to be high among young patients [15, 19]. For example, in Uganda, Kenya and Tanzania it was reported that, most patients with BC were aged 50 years or younger and most of them 67.4%, 41.1% and 76.9%, respectively had tumour grade 3 [11, 13, 15]. However, these findings differ from results of studies conducted in developed countries. It has been reported that, patients with BC in developed countries most of them have low tumour grade as well as low tumour stage at presentation. In two studies one from Ireland and another from Norway they reported that 15.2% and 47%, respectively of the patients had grade 3 [20, 21]. Also in the study that was done by Adjei et al which included patients with BC from Norway and Ghana, it was found 57% of cases from Ghana had grade 3 and only 30% of the cases from Norway had grade 3 [17].

The difference in the histological grades from studies done in different settings is mainly due to the difference in the trend of breast screening practices for the different settings. It is clearly known that, delaying in screening for BC contributes largely to early detection of the disease. This leads to detection of the disease while at advanced stage with high grade especially in developing countries [22]. Negative

health seeking behaviour and lack of awareness and low and/or lack of knowledge on BC in developing countries, remain the major factors for the available high prevalence of high grade BC in the literature. The reason why the prevalence of BC in patients aged 50 years or younger in developing countries is higher compared to western countries could be attributed to natural history of the disease (tumour biology) which needs further investigation to understand [23]. This may include genetical screening for the carrier status of both BRCA1and BRCA2 genes mutation and also including detailed analysis of the risk factors for BC while comparing the pre-and post-menopausal females at risk of developing BC.

Regarding histological subtypes of BC in our study; IDC-NST was present in the varsity majority of the patients (68.9%). This is similar to the reports from both developed and developing countries. In the previously done studies in Tanzania, Uganda, Nigeria and Norway IDC-NST was reported in 88.6%, 89.6% and 78%, 95% and 81.4%, respectively [12, 14, 15, 20, 24]. This is because BC commonly develops initially from the ducts and occasionally it may develop from lobules for example and also its pathogenesis may be coupled by formation of different morphogenesis such as formation of mucin, papillary mesenchymal components and metaplastic changes among many others [20]. ILC in our study was the second in terms of prevalence which also similar to many other previously done studies. Four percent, 6.3% and 2.9% of ILC were reported in Uganda, Norway and Tanzania [14, 15, 17]. However, these proportions are very low when compared to 22.3% of ILC was found in the present study. This variation may be due to difference in diagnostic methods used in the different studies, difference in experience in reporting the histological tissue sections and possible tumour biology of BC in the different countries. The prevalence of other histological types in our study was as low as in many previously done studies.

Expression of hormonal receptors varies greatly from one study and another in the same setting and sometimes between different races or geographical areas. The ER expression of 53.4% in the present study was quite higher than 32.7% and slightly lower than 60% which was reported by Galukande et al and Roy et al from Uganda [14, 15]. In Tanzania the expression of ER in patients with BC was also reported to vary across different studies at different periods. For example, Mwakigonja et al, Mbonde et al and Rambau et al reported 43%, 33% and 32.7%, respectively [10–12]. These findings indicate how the prevalence of ER expression vary across different studies within the same setting. In studies which were done among African women with BC residing in USA and Britain it was reported that the prevalence of expression of ER was 61% and 66%, respectively [20, 25]. Adjei et al rreported 76% and 85% expression of ER in a study which was done to compare the expression among Ghanaian and Norwegian women with BC [17]. The major reason for the existing difference in expression is the issue of preanalytical phase handling of the biopsies. These differences could be due to the different study designs, sample size used and settings. The time taken before fixing of the biopsies, the time taken for tissue fixation and ratio of the fixative to the volume of the specimen, have been to contribute greatly to affecting the staining ability of the IHC antibodies including ER.

Of the HRs, ER is usually expressed higher than PR. Most studies have reported PR expression in tissue blocks to range from 13.9–61.3% of both primary and metastatic breast cancers [26, 27]. This observation was also found in the present study. The 46.6% prevalence of expression of PR in our study

was similar to 42.3% reported by Rambau et al in Tanzania but higher than 10%, 31.8%, 5.8% and 26.4% expression of PR which was reported in Kenya, India, Uganda and Tanzania, respectively [11–13, 15, 28]. Adjei reported a higher prevalence of expression of PR of 65% in Ghanaian women with BC. The same study also reported that, PR expression in Norwegian women was 82% [17].

Endocrine therapy (ET) is well established for early and advanced BC with treatment decisions currently based on the semiquantitative and IHC assessment of HRs expression on histological biopsies. Imperative use of ET in patients with BC has been shown to have no value in improving treatment outcomes of the patients. Some studies have even reported possible worse prognosis that are more likely to result from imperative use of ET [29]. The prognostic role of ER and PR has been reported to vary. In a study which was done by Purdie et al in United Kingdom (UK), it was found that, PR was a potential predictor of overall survival, breast cancer-specific survival and disease-specific survival. The study reported that, patients with negative PR were 3.24 times more likely to die than the ones in whom PR was positive and the difference was statistically significant (95% CI 2.42–4.34, $p < 0.0001$) [29]. Furthermore, the same study found that, the prognosis of the patients was poor even in cases who are ER positive provided that PR was negative.

BC cases in whom ER is negative but PR is positive are exceptionally rare and are said to comprise 0.3% [29]. This suggests that the assessment of PR expression in ER-negative tumours to identify those that might still benefit from ET may not be justified [30]. ER, PR and co-expression of ER and PR in this study was found to increase with increase in age of the patients. For all the biomarkers were more expressed in patients who were aged 50 years or more unlike those aged 50 years or younger. These data are all in keeping with previous studies [31] and indicate that PR-expressing tumours are more common in post-menopausal women with low-grade, ER-positive breast cancers who, generally, have a good prognosis. Additionally, age was the potential predictor of ER and PR but not co-expression of two biomarkers. These findings may help to provide insightful information regarding preferential use of imperative use of these markers in postmenopausal women with BC unlike premenopausal cases.

Many studies have reported that younger age is an independent factor for poor prognosis in breast cancer [8, 32, 33]. This fact was not different in the current study, where among all the patient characteristics, it was only young age that showed a statistical significance with expression of ER, PR hormones and triple negative breast cancer. Post menopause patients (50 years and over) were 0.39 times less likely not to have triple negative breast cancers, but 3.7 and 3.9 times more likely to show expression of negative ER and PR receptor hormones respectively (Tables 4). These findings support the notion that age at diagnosis should be taken into account when studying effects of breast cancer risk factors [34]. This study finding is consistent with findings by Fletcher, which reported that age may play a role in increased risk of developing triple-negative breast cancer. Premenopausal women have been found to develop triple-negative breast cancer more often than postmenopausal women [35]. In another similar study conducted in China revealed that women aged forty years and below were more likely to be PR positive compared to those aged above 40 years [36] which is in agreement with the current study.

HER2-positive BC has better prognosis than HER2-negagive BC. Patients with BC who are pre-menopausal are more likely to be HER2-positive than post-menopausal ones. Also Purdie et al reported that, ER-positive BC cases are more likely to be HER2-positive than PR-negative BC cases [29]. The expression of HER of 18.4% in the present study was similar to 18.5% which was reported in Brazil and lower than 40.7%, 24%, 23.1% and 22% that were reported in India, Ghana, Tanzania and Uganda [11, 15, 17, 28]. Studies have shown that, expression of HER in both African American and African patients with BC is higher compared to Caucasians. For example, in the study done by Adjei et al which was comparing expression of breast markers between Ghanaian and Norwegian women with BC, it was found that, the expression of HER2 in Ghanaian was 24% whereas in Norwegian patients HER2 expression was found in 14% [17]. Purdie et al also reported 13.9% of HER2 expression among patients with BC. The difference could be due to the difference in methodology. Studies that involve the use of fluorescent in situ hybridization (FISH) technique in deciding whether the equivocal cases (2+) are truly positive, have reported lower prevalence of HER expression than studies which don't involve FISH method.

Prediction of expression of HER2 by age, tumour grade and histological type in this study was not having any statistical association. However, the expression of HER2 in this study was higher in patients with ≤ 50 years than those with > 50 years (68.4% versus 31.6%). Also expression of HER2 in this study was increasing with increase in the tumour grades but without statistical association (Table 6). Although most of studies have reported that patients with BC aged less than 50 years tend to have a higher expression of HER2 than those with 50 years or over, however, some studies have shown contradicting results. For example, Arias et al reported that, there was 57.7% and 41.1% of postmenopausal and premenopausal patients with BC that were expressing HER2 and the difference in expression of the biomarker was not statistically significant ($p = 0.065$) [37]. Mwakigonja et al reported that, the expression of HER2 in the patients with BC aged ≤ 55 years was 41% compared to 10% for those aged > 55 years and the difference was statistically significant ($p = 0.079$) [12]. in another study done by Marketa et al it was also found that the expression of HER2 in patients with BC aged 20–39 years was higher than those aged more than 50 years [38].

HER2 expression in our study was increasing with increase in the tumour grades. However, the increase in expression was not statistically significant (Table 6). This is in line with the finding in the study done by Rao et al [39] in India who also reported that, HER2 overexpression was higher among cases with grade 3 than those with either grade 2 or 1 but the difference in expression was statistically significant ($p < 0.05$). Parise et al [40]also reported that, the risk of patients with BC that were expressing HER2 in the study was 3.9 compared to 2.7 of the ones who were not expressing HER2.

TNBC carries the worst prognosis of all the molecular subtypes of BC (Luminal A, B, C, HER2 and TNBC). TNBC is an aggressive disease, recurring and metastasizing more often than other kinds of breast cancers [41]. Patients with BC who fall in this category of being triple negative (TN), they do not respond to either hormonal therapy or targeted therapy such as trastuzumab which usually is of prognostic value for those patients with BC who can express HER2. The prevalence of TNBC in this study of 37.9% was almost similar to 36%, 34% and 38.4% which were previously reported in Uganda and Tanzania [11, 14,

15]. Slightly higher prevalence of TNBC of 41%, 45.6% and 50% was reported previously in Uganda, Tanzania and India [12, 39, 42]. The reasons for higher rates of TNBC are not well understood, however, partly they may include genetical predisposition and difference in methodology used in determining expression of the breast markers. Adjei et al reported that, the prevalence of TNBC in Ghanaian women with BC was 3 times more than Norwegian women with BC (22% versus 7%) [17]. This finding indicates the fact that, there are many patients who are TN from the African race unlike in the population of the whites.

Age was the potential predictor of TNBC in this study. Patients aged 50 years or over, were 4.4 times more likely to have TNBC than those aged more than 50 years and the difference was statistically significant ($p = 0.009$). This observation is similar to the findings in the study by Jiayu et al which was done in China which found that, the prevalence of TNBC in patients aged ≤ 40 years was 13.8% compared to 7.1% found in patients with > 40 years but the difference in having TNBC between the two age groups was not statistically significant ($p = 0.443$) [43]. Marketa et al in Czech Republic also reported that 66% of the cases with TNBC were in the age group 20–29 years [38] In another study which was done in United Kingdom (UK), it was found that, patients with TNBC were carrying the worst prognosis than the rest of the molecular subtypes [40].

Tumour grading in our study showed no statistical association with occurrence of TNBC in the cases. However, as the tumour grades were increasing, the number of cases with TNBC was also increasing. This observation is similar to the finding in the study of Rao et al which reported that, patients with high grade (grade 3) were found to have TNBC compared to the rest of the grades and the difference was statistically significant ($p < 0.05$) [39]. On the other hand, histological types were not the predictor of TNBC in this study despite that there more IDC-NST type cases that had TNBC (Table 7).

Limitations

We were unable to control issues related to pre-analytical phase including fixation time, cold ischemic time could, tissue storage and thickness of the sections during grossing altogether may affected the quality of immuno-staining results. Because of the nature of the study being retrospective, we were not able to obtain other variables such as tumour stage, lymph node status tumour size among many others which would have increased the power of the methodology. Additionally, financial constrains including lack of proliferation biomarkers such as Ki67 would have helped to perform molecular subtyping of the BC in our study.

Conclusion

The varsity majority of the patients with BC in our study were aged 50 years or younger and they had IDC-NST with grade 2. HRs expression was higher in postmenopausal cases than in premenopausal cases. On the other hand, HER2 expression and TNBC were found more in premenopausal patients than

postmenopausal ones. Age was a strong predictor for expression of ER, PR and occurrence of TNBC among the cases included in our study.

List Of Abbreviations

BC-Breast cancer, ER-Estrogen receptor, PR-Progesterone receptor, HER2-Human epidermal receptor-2, HR-Hormonal receptor, TN-Triple negative, TNBC-Triple negative breast cancer

Declarations

Acknowledgement

We thank Mr. Peres for performing IHC staining and also Mr. Noel Namuhani for working with statistics.

Authors' contributions

AMT: Designed the research and contributed to the conception of the study, literature search and data collection and writing the first draft of the manuscript. EO, SK and LAO: Supervised the research and revised the manuscript critically. JJY: Organized, revised the manuscript critically and worked on statistics. All authors read and approved the final manuscript to be submitted.

Funding

This research did not receive fund from any source.

Availability of data and materials

The datasets used during in this study are available from the corresponding author and they may be provided when requested.

Ethics approval and consent to participate

All procedures performed in this study were in accordance with the ethical standards of the Ethics Committee of Makerere College of Health Sciences (MakCHS).

Consent to publish

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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Figures

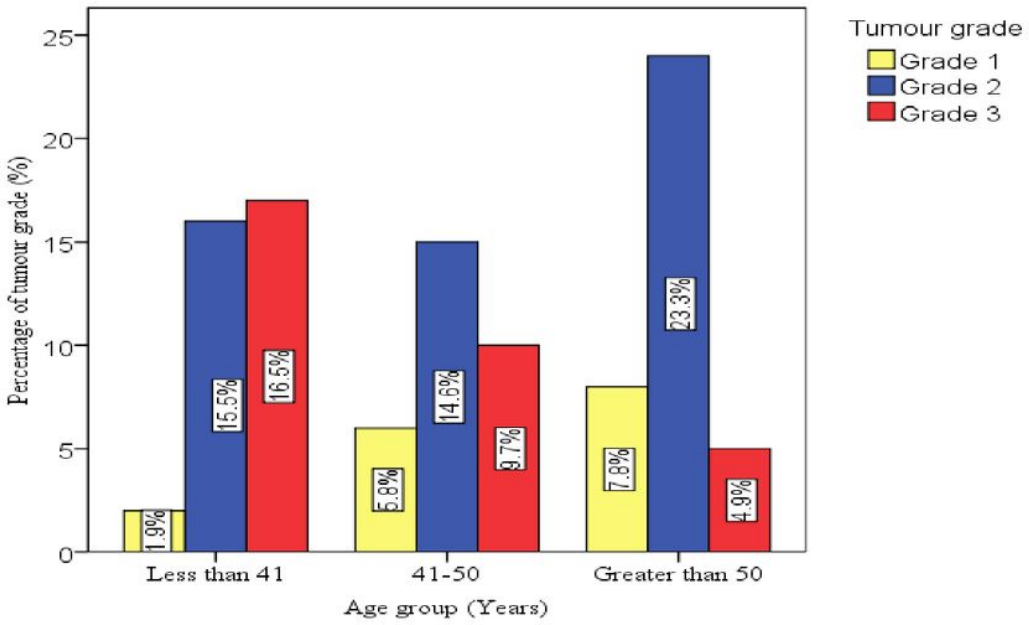

Figure 1

Figure 1

Histological grade of cancers among the different age groups of the patients

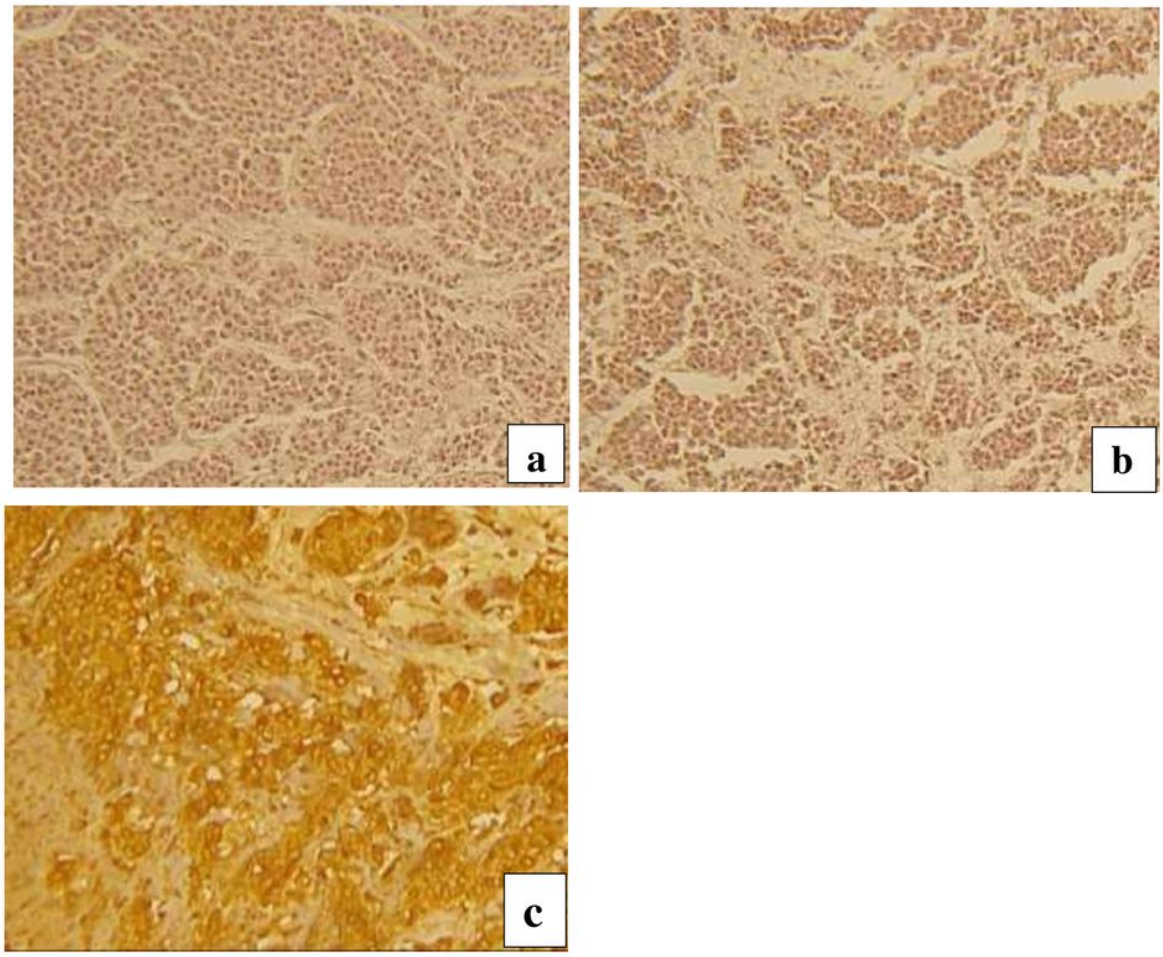

Figure 2

Figure 2

a- Intranuclear diffuse staining of the tumour cells with ER in a case of IDC-NST(IHC staining, x40), b- Intranuclear diffuse staining of the tumour cells with PR in a case of IDC-NST(IHC staining, x40), c- Complete nuclear membrane of the tumour cells with HER2 protein in a case of IDC-NST (IHC staining, x40)