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Expression Patterns of ER, PR, Her-2/neu and p53 in Association with Nottingham Tumor Grade

A retrospective hospital-based study

***Kamoru A. Adedokun,[1] Waheed A. Oluogun,[2] Musiliu A. Oyenike,[3] Sikiru O. Imodoye,[4] Lukman A. Yunus,[2] Ismaila A. Lasisi,[5] Ibrahim O. Bello,[6] Ramat T. Kamorudeen,[7] Saheed A. Adekola[8]**

[1]Department of Immunology, Roswell Park Comprehensive Cancer Center, Buffalo, New York, USA; [2]Department of Morbid Anatomy and Histopathology Osun State University Teaching Hospital (UNIOSUNTH), Osogbo, Nigeria; [3]Department of Medical Laboratory Science, Ladoke Akintola University of Technology, Ogbomosho, Oyo State, Nigeria; [4]Department of Oncological Sciences, Huntsman Cancer Institute, University of Utah, Salt Lake City, Utah, USA; [5]Laboratory Unit, Health Centre, Osun State Polytechnic Iree, Osun State, Nigeria; [6]Department of Biological Sciences, Southern Illinois University at Edwardsville, Edwardsville, Illinois, USA; [7]Department of Public Health, University of South Wales, Pontypridd, UK; [8]MBM Molecular Laboratory, Dubai, United Arab Emirate.
**Corresponding Author's e-mail: adeolokun@yahoo.com*

Abstract

Objectives: Histological grading has been an integral part of cancer diagnosis for a long time. Recent molecular studies show that breast cancer is a heterogeneous disease, and several molecular changes may accumulate over time to influence treatment response. As a result, employing reliable molecular biomarkers to monitor these modifications may help deliver personalized treatment. However, this may be unrealistic in the resource-limited parts of the world. Thus, we studied the expression pattern of hormone receptors and p53 tumor suppressor using immunohistochemistry (IHC) in breast cancer

(BC) compared to the traditional tumor grade. ***Methods:*** Two hundred and five (*n* =205) cases were investigated. The Modified Bloom-Richardson score system was adopted in grading the tumors. Tissue sections of the cases were stained with specific primary antibodies (at dilutions of 1:60 for estrogen (ER) and progesterone receptors (PR), 1:350 for human epidermal growth factor (Her-2/*neu*), and 1:50 for p53. The Chi-square test was used to determine the association between the tumor grade and IHC markers. ***Results:*** Invasive ductal carcinoma of no-specific type (190 cases;92.7%) was predominant. Grade II tumor (*n* =146; 71.22%) was the most frequent. Hormone receptors (ER+; *n* =227 and PR+; *n* =145) had 62.0% and 70.7% positive cases; 34.2% (*n* =70) were positive for Her-2/*neu*, while 76.1% (*n* =156) were positive for p53. We observed strong associations between Nottingham grade and expression patterns of ER ($P < 0.01$), PR ($P < 0.001$), Her-2/neu ($P < 0.001$), and p53 ($P = 0.001$). ***Conclusion:*** Nottingham grade has a high degree of concordance with the patterns of expression of hormone receptors, Her-2/neu, and p53, suggesting that it may play an important role in connection with the predictive and prognostic biomarkers for BC.

Keywords: Breast cancer, Her-2/*neu*, hormone receptor, Nottingham grade, p53 mutation.

Advances in Knowledge

- Grade II tumors displayed higher levels of ER and PR expression than grade III tumors, indicating that as the disease progresses, the proportion of cells expressing ER and/or PR steadily declines.
- Similarly, we observed higher HR positivity than in many black populations, including Guinea, Ghana, South Africa, and Mali emphasizing potential identifiable intra-racial factors influencing the diverse variation.
- Some patients had higher grades in the Her-2/*neu*+ expression group than in the Her-2/*neu*- expression group but lower in ER+ and PR+ expressions compared to ER- and PR- of the highest grade III.
- We found a higher Her-2+ than the majority of previous studies, but most of these cases co-expressed HR+ with Her-2/neu- rather than Her-2+ tumors, indicating that the cancer cells are responsive to hormone treatment, have a better prognosis, and are less aggressive, contrary to the common opinion that black population has aggressive breast cancer presentation.
- The proportion of TNBC patients was rather low, implying that hormone therapy or targeted therapies targeting at Her-2 would benefit the majority of our cancer patients.

Application to Patient Care:

- Our study shows that cancer phenotype can exhibit location-dependent variations due to several factors including genetic predisposition, lifestyle, and environmental influences.
- We observed that the underlying factors for regional, ethnic or racial variation can impact the expression patterns of various biomarkers.
- As a result, this study implies that understanding regional variations in cancer phenotype and biomarker expression patterns, as well as tumor grade, can help guide personalized treatment decisions, optimize therapy selection, and perhaps improve patient outcomes.

Introduction

Cancer continues to be one of the deadliest noncommunicable diseases worldwide.[1] Although the literature shows that BC is more common in developed countries, a recent GLOBOCAN estimate shows that Africa constitutes a nerve-racking proportion of BC deaths, possibly due to poorer prognosis and limited access to appropriate diagnosis and treatment.[2] Before the advent of molecular diagnosis, most cases of BC were solely diagnosed using histological methods. Yet, the histological method is still commonly and exclusively used, especially in low-resource settings in many African countries.[3]

In this new genomic era, molecular markers are gaining wide acceptance as sensitive and inclusive methods to understand the behavior of advanced cancers. Specifically, hormone receptors, p53, Ki67, and human epidermal growth factor receptor 2 (Her-2/neu) are used for the diagnosis, classification, prognosis, and prediction of response to therapy in BC; even so, histological assessment is used primarily.[4] Each of these biomarkers is important in diagnosing BC and may sometimes correlate with other disease diagnostic indicators. Overexpression of Her-2/neu has been linked to a higher histological grade, increased tumor size, the number of affected lymph nodes, p53 mutation, and lower ER expression (or even ER expression in some cases).[5] Similarly, ER and PR patterns have been linked to BC grade, potentially influencing treatment options.[6,7] Furthermore, a mutation in the p53 gene, a tumor suppressor gene, represents a genetic predisposition to cancers [8] and has been associated with tumor aggressiveness [9], making them a possible indicator of histological grade.

Meanwhile, histological grade enables a description of a tumor's level of aggressiveness and is regarded as a forerunner for morphological evaluation of tumor biological characteristics.[10] According to a study on gene expression, histological grade reveals information about the molecular makeup of BC in addition to tumor size or lymph node involvement.[11] Furthermore, evidence from genome-wide microarray-based expression profiling elucidates many characteristics of tumor biology in BC, adding to the evidence that the biological features revealed by histological grade are critical in determining tumor behavior. [10]

The investigation of the connection between histological grade and molecular biomarker expression patterns is thought to add to the body of diagnostic knowledge, particularly in the areas where molecular testing is currently lacking. Even though they are complementary, more research is needed to determine the magnitude of the relationship between traditional tumor grading and the more contemporary IHC methodologies, particularly regarding expression patterns. This attempt may highlight the importance of histological grade in low-resource settings as a low-cost, easy, accurate, and validated approach to diagnosing BC. In the present study, we investigated the frequency and patterns of expression of some clinically significant molecular markers in patients with BC. We explored the link between the biomarkers' expression patterns and histological tumor grade to determine their role in disease diagnosis.

Methods

Study design and patients

This investigation was a hospital-based retrospective study. It involved archival tissue blocks and records of female patients older than 18 years referred to LAUTECH Hospitals in Osogbo and Ogbomosho, Osun and Oyo States, respectively (at the time of the investigation). The study included patients on record between 2005 and 2014 for breast biopsy or surgery diagnosed with BC in their pathology reports.

Slide preparation

Tissue blocks were retrieved and new thin sections of about 3µm were made using rotary microtome from formalin-fixed paraffin-embedded blocks following a previous method.[3]

Clinicopathological features

Data vis-à-vis; age, histological grade, nuclear grade, tumor size, and lymph node involvement were extracted from patients' records.

Tumor classification

Histological classification of the breast tumor was made following World Health Organization (WHO) guidelines. Tumor grading was done using Nottingham modification of the Scarff-Bloom-Richardson (SBR) grading system. Tumor staging was done using the TNM system adopted by International Union against Cancer (UICC) and the American Joint Committee on Cancer and End Results Reporting (AJC)[12].

Immunohistochemical assessment

All samples were evaluated by immunohistochemical (IHC) staining under the direct supervision of a Chief Histopathology Scientist and reported by two different Consultant Pathologists, which were then compared in a blinded fashion. The procedures for IHC staining were performed using the primary antibody specific for ER (ER6F11) (Dako), PR (Dako), Her-2/*neu*is (ERBB2) (Dako), and p53, Do-7 (Santacruz) at the Breast Cancer Laboratory Medical Genetic and Bioethics Research Unit, Institute for Advanced Medical Research and Training (IMRAT), University College Hospital, Ibadan. The sections were exposed to the primary antibody (dilutions of 1:60 for ER and PR, 1:350 for Her-2/*neu*, and 1:50 for p53 for one hour). Negative and positive controls were performed by including the control tissues specified by the antibody vendors, respectively.

Scoring of ER and PR status

The scoring was performed using the modified immunohistochemical score ("Quickscore"), a modified semi-quantitative assessment method by Allred [13]. Nuclear staining intensity was scored from 0 to 3+ in combination with the proportion of cells involved to get a range of 0–7 as the final score for ER and PR positivity [Figure 1]**.**

The criteria used are explicitly described as follows: "Quickscore" determines the percentage or range of stained cells from 1 to 4 and overall intensity from 1 to 3. The scores are added to give a total maximum score of 7 (Table 1). Chances of benefit from Hormonal Therapy were classified as

follows: 0–1 = No effect; 2–3 = Small (20%) chance of benefit; 4–6 = Moderate (50%) chance of benefit; 7 = Good (75%) chance of benefit.

Scoring of Her-2/neu status

For Her-2/*neu* expression, the only membrane staining pattern was scored from 0 to 3+, where 0/1+ indicates negative, 2+ stands for equivocal, and 3+ means positive following the standards outlined by Ellis et al.[14].

The criteria used are explicitly described as follows: Negative (0 scores): Membrane staining <10% of the tumor cells, or no staining detected. Negative (1+ score): Membrane staining detected in >10% of the tumor cells or faint staining detected. The stain was observed only in some parts of the membrane. Equivocal (2+ score): A weak to moderate complete membrane staining was detected in >10% of the tumor cells. Positive (3+score): A strong complete membrane staining was detected in >10% of the tumor cells. The molecular classification was based on the positivity and negativity of ER, PR, and Her-2/*neu* [Figure 1].

Scoring of p53 status

For p53 expression, the nuclear staining pattern was scored from 0, 1+, 2+ to 3+, the numbers 0, 1+, 2+, and 3+ were used to describe the intensity of the staining of the p53 protein in the cells (reported by Bergh)[15]. The degree staining was used to determine whether the p53 protein is overexpressed or not. The numbers 0 and 1+ indicated negative staining, while the numbers 2+ and 3+ indicated positive staining, as depicted in Figure 1c. The p53 protein is considered negative if it is not overexpressed or mutated.

Statistical analysis

Data obtained were reported in percentage and proportion using descriptive statistics. No calculation of sample size was done, and all cases with complete information were entered into the study. The Chi-square test was used to determine the association between histological tumor grades (I, II, and III) against the expression patterns of individual selected molecular markers (for ER/PR expression, Her-2/*neu* overexpression, and p53 mutation). A value of $P < 0.05$ was considered statistically significant.

Ethical approval and consent

Ethical approval was obtained from the LAUTECH Health Research Ethics Committee. This study posed no risk to the participants and the community at large. Data generated were made confidential, and no patients' names were recorded.

Results

This was a hospital-based retrospective study involving biopsy/surgical cases of BC recorded over 10 years. Two hundred and five (n = 205) cases were investigated for IHC markers—hormone receptors (estrogen receptor, ER and progesterone receptor, PR), human epidermal growth factor receptor (Her-2/*neu*), and p53 immunomarkers.

Age distribution

The age range was 21 and 87 years (mean = 49.30 years) of the total cases. The peak age of this incidence was 50–59 years.

Laterality

By laterality, the records showed that BC occurred at nearly the same rate between the left (n = 103 cases; 50.2%) and the right (n = 102 cases; 49.8%) breast sides among those with complete records.

Histological type

The most frequent histological phenotype of female BC recorded was infiltrating ductal carcinoma (IDC) (190 cases: 92.7%). Other less frequent types were invasive lobular carcinoma (ILC) (8 cases; 3.9%) and medullary carcinoma (3 cases; 1.5%), while the rare frequent phenotypes were mucinous carcinoma, carcinosarcoma, metaplastic carcinoma, and poorly differentiated carcinoma had 1 case each (0.49%), respectively.

Tumor grade

Using Nottingham modification of the Bloom-Richardson system, the frequency distribution by tumor grade was recorded [Table 2].

Tumor size

All the cases had specified tumor sizes ranging between 1–22 cm in the widest diameter (mean = 5.8 cm). The frequency distribution is shown in Table 2.

Lymph node metastasis

Table 2 also illustrates the degree of lymph node (LN) involvement. LN biopsy was reviewed in the record for a possible note of metastasis in individual cases. The frequency distribution is shown in Table 2.

Nottingham prognostic index

The Nottingham Prognostic Index (NPI) traditionally involves a combination of the assessments of nodal status, tumor size, and histological grade for its potential survival outcome. It is based on a recent prognostic scoring, namely, NPI-I (excellent) ≤2.4; NPI-II (good) >2.4 but ≤3.4; NPI-III (moderate) >3.4 but ≤5.4; and NPI-IV (poor) >5.4 [16]. Our data showed that out of 205 cases, 63 cases (30.7%) indicated a good prognosis, 100 cases (48.7%) signified a moderate prognosis, and 42 cases (20.5%) showed a poor prognosis.

Immunohistochemical profile

Two hundred and five female (*n* = 205) BC cases were processed and stained for ER, PR, Her-2/*neu* antigen, and p53 positivity.

Two hundred and five female (*n* = 205) BC cases were immunostained for ER and PR. One hundred and twenty-seven cases (*n* = 127; 62.0%) were positive, ER+, while 78 cases (38.0%) were ER-. One hundred and forty-five cases (*n* = 145; 70.7%) were PR+, while 60 cases (29.3%) were PR-. The intensity and its score are shown in Table 3A and Figure 1.

Two hundred and five cases (*n* = 205) were immunostained for Her-2/*neu.* Seventy cases (*n* = 70) 34.2%) were Her-2/*neu*+**,** while eighty-one cases (*n* = 81; 39.5%) were Her-2/*neu*-. Fifty-four cases (*n* = 54; 26.3%) were equivocal. For the equivocal result, the stains were not furthered with fluorescent in situ hybridization (FISH) due to limited funding but considered Her-2/neu-.

The staining intensity and the score for Her-2/*neu* are shown in Table 4 and Figure 1b.

Two hundred and five cases (n = 205) were analyzed for p53 immunostain. One hundred and fifty-six cases (n = 156; 76.1%) were p53+, while 49 cases (23.9%) were p53-. The staining intensity and the score for p53 mutation are shown in Table 4 and Figure 1c.

Immunohistochemical profile and Nottingham tumor grade

We observed associations between the expression profile of hormone receptors (ER and PR), Her-2/*neu*, and p53 compared to the Nottingham tumor grade. The pattern of expression in ER (positivity) showed a significant difference ($P < 0.01$) compared to the distribution of patients according to tumor grades, in the same way as PR positivity ($P < 0.001$). Likewise, the pattern of Her-2/*neu* expression (connecting positive, negative, and equivocal staining distribution among the incident cases) showed a significant difference ($P < 0.001$) compared to the Nottingham tumor grade pattern. Also, the association ($P = 0.001$) between the p53 expression pattern and the Nottingham tumor grade pattern was observed.

Based on the results provided above, we classified the breast cancer subtypes along with their proportions in this study into the following groups:

ER/PR positive, Her-2/neu negative cases were 110 (53.6%); This subtype was characterized by the presence of estrogen and progesterone receptors but the absence of Her-2 overexpression through ER+/PR+, Her-2-; ER-/PR+, Her-2- and ER+/PR-, Her2-.

ER/PR positive, Her-2/neu positive cases were 48 (23.4%); This subtype was defined by the presence of both estrogen and progesterone receptors, as well as Her-2 overexpression through ER+/PR+, Her-2+: ER-/PR+, Her-2+ and ER+/PR-, Her-2+.

ER/PR negative, Her-2/neu positive cases 22 (10.7%); This subtype was identified by the absence of estrogen and progesterone receptors but the presence of Her-2 overexpression through ER-/PR-, Her-2+.

Triple-negative cases were 25 (12.2%): This subtype was specified by the absence of estrogen and progesterone receptors, as well as Her-2 overexpression through ER-/PR-, Her-2- and ER-/PR-, Her-2-.

Discussion

In this study, we retrospectively investigated 205 BC cases in western Nigeria for hormone receptor (HR) expression (HR: estrogen receptor [ER] and progesterone receptor [PR]), human epidermal growth factor receptor (Her-2/*neu*), and p53 expression profile in terms of pattern and frequency. We explored the expression patterns of these biomarkers in connection with the tumor's aggressiveness using the conventional Nottingham grade.

From our findings, the molecular characteristics of the tumor showed that ER and PR were positive in 62% and 70.7% of the total recorded cases, respectively. There were associations between ER and PR's expression patterns and the tumor grades' frequency. This is following the report on Polish women, which showed an association between tumor grades and HR positivity.[17] The present study showed that grade II tumors had a higher ER and PR positive frequency than grade III. Meanwhile, a previous report [18] indicated that the number of cells expressing ER and/or PR gradually decreases with disease progression. This was substantiated by the report of Badowska-Kozakiewicz *et al.* [17], which showed an inverse correlation between ER expression and the size of the primary tumor. In specific terms, in addition to positively predicting therapeutic outcomes, estrogen receptor α (ERα) is believed to inhibit epithelial-mesenchymal transition by promoting epithelial phenotype and preventing tumor invasion in breast cancer.[19] We observed higher HR positivity than in many African populations, including Guinea [20], Ghana [21], South Africa [22], and Mali [23]. Although there is no specific identifiable factor influencing the diverse variation from one population to another, a previous study suggested that small sample sizes recruited for studies across African countries could be a possible reason.[20] Even though our study showed higher HR positivity compared to a study of a considerably similar population in Nigeria, where a multicentric study involving 507 patients was previously carried out.[24] Conversely, our data are in tandem with reports involving BC patients in Western countries [6] and the Saudi population [7], where high HR is also documented. Potemski *and co*workers [25] reported related results and revealed that the higher the level of receptor expression, the lesser the mortality. In line with their observations, our study also showed that the majority of our incident cases had a moderate prognosis with high HR positivity and lower tumor grades, indicating a possible association between HR expression and tumor grade.

In addition, regarding the Her-2/neu expression pattern in this study, some (39.5%) of the cases were negative and were more than the positive (34.2%) outcome, with an unexpected increase in Her-2+ proportion than many reported cases. Equally, patients were classified histologically as having higher grades in the Her-2/*neu*+ expression group than in the Her-2/*neu*- expression group but lower in ER+ and PR+ expressions compared to ER- and PR- of the highest grade III [Table 4]. In agreement with our study, Arafah [7] reported that the histologic grade of BC was significantly associated with both ER and PR expressions but, in turn, found a negative correlation between HR and Her-2/*neu* stains. Also, Aman *et al*. [26] recently associated overexpression of Her-2/*neu* with higher Nottingham grade in an Ivorian population. Again, in the literature, concurring with the present study, a study involving the Chinese population reported a link between Her-2/*neu* overexpression and a higher histological grade with a higher incidence rate of infiltrating ductal carcinoma, among many other factors.[8] Although the majority (92.7%) of the incident cases in this study were infiltrating ductal carcinoma, which is in line with the study of Ding and his colleagues[9], our analysis also showed a strong association between histological grading and the pattern of expression of Her-2/*neu.* However, our observations indicated that Her-2/neu overexpression is linked to the aggressive forms of BC, as previously reported by Arteaga and his colleagues.[27]

Moreover, to better understand the therapeutic benefits for the patients, we classified the patients based on histological phenotypes of the hormone receptor and Her-2/neu expression patterns. Most notably and in agreement with the report of Gago et al.[28], the majority of our breast cancer patients co-express HR+ with Her-2/neu- rather than Her-2+ tumours, indicating that the cancer cells are responsive to hormones such as estrogen and progesterone, better prognosis and also preventing tumour aggressiveness. On the other hand, among the Her-2+ category, a smaller number of ER/PR-Her-2/neu+ was observed representing breast cancer cases where both the ER and PR are negative, while the Her-2/neu is overexpressed. This subtype is commonly known as hormone receptor-negative, Her-2/neu-positive breast cancer. It suggests that the cancer cells do not respond to hormones and have an overexpression of the Her-2/neu gene. More importantly, triple-negative breast cancer (TNBC) is a vastly diverse group of tumours, which represents 15-20% of all breast cancer cases Kummel et al.[29]. The proportion of the TNBC in our study is relatively small suggesting an advantage against the studied population. Meanwhile, TNBC is the most difficult to treat among all breast cancer phenotypes because the common hormonal therapy used for the majority of breast

cancer subtypes is treatment-refractory for TNBC. On the hand, TNBC is often treated in its early stages with surgery, radiation, and chemotherapy.

Furthermore, most of our investigated cases (70.1%) were p53 positive, and there was a strong association between the p53 expression pattern and the Nottingham tumor grade. Consistent with other studies [5, 30], our findings, therefore, implied that the p53 positivity may have a connection with tumor grade in terms of the frequency of the incident cases. Patients in the p53+ expression group were classified histologically as higher grades than those in the p53- expression group, similar to the previous report [24] and corresponding to the Her-2/neu expression pattern in this investigation. Shokouh *et al*.[5] earlier demonstrated that p53 expression had a significant association with the grade of BC. Various reports have outlined the functional role of p53 in the progression of BC. Mechanistically, p53 activates protein transcriptions involved in the DNA repair mechanism. However, if the mechanisms fail due to a defective p53, aberrant cells may proliferate uncontrollably, leading to cancer [31.] A report shows that tumors with p53 mutations are more likely to be aggressive and resistant to chemotherapy and radiotherapy.[29] In other words, p53 immunoreactivity is linked to histologic grade, particularly a tumor's high mitotic index.[8]

Limitations of the study

According to the Her-2 testing guidelines of the American Society of Clinical Oncology and the College of American Pathologists (ASCO/CAP), breast cancer that is reported 2+ equivocal by IHC should be followed up with in-situ hybridization (ISH) testing to confirm the cases for possible gene amplification. However, the current study is limited by the inability to verify the negative (2+ score) results with fluorescence in situ hybridization (FISH), and thus considered negative. This could have an impact on the negative result value.

Conclusion

Our observations suggest that expression patterns of PR, ER, Her-2/*neu*, and p53 were influenced by the tumor grade (level of aggressiveness). In other words, there is an association between the tumor grade and expressions of PR, ER, Her-2/*neu*, and p53, which suggests that the Nottingham grade is still relevant as a reliable prognostic marker for BC.

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Conflicts of interest

The authors declare no conflicts of interest.

Authors' Contribution

KAA, WAO, and MAO were involved in conceptualization and design of the study. WAO, MAO, LAY, and RTK collected the data. KAA and SOI analyzed and interpreted the results. KAA drafted the manuscript. KAA and SOI revised the manuscript. KAA, SOI, IAL, IOB and SAA joined hands in the literature search. WAO carried out clinical studies. All authors approved the final version of the manuscript.

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Table 1: Scoring Guideline ("Quickscore") for ER and PR

Proportion score	Observation	Intensity score	Observation
Zero	Zero staining	Zero	No staining of any nuclei even at high magnification
1	1 – 25%		
2	26 – 50%		
3	51 - 75%		
4	76 – 100%	1	Weak staining (only visible at high magnification)
		2	Moderate staining (Readily visible at low magnification)
		3	Strong staining (strikingly positive even at low magnification)

The score for intensity is then added to the score for proportion, giving a range of 0-7.

Table 2: Frequency distribution of tumor grade, size, and lymph node involvement in female breast cancers

	Tumor index	Frequency(%)
Tumor grade[α]	I (Low)	16 (7.80)
	II (Intermediate)	146(71.22)
	III (High)	43 (20.98)
Tumor size[β]	pT1	18 (8.78)
	pT2	106(51.71)
	pT3	81(39.51)
Lymph node status[ɤ]	pN0	156(76.1)
	pN1	46(22.44)
	pN2	3(1.46)

"ɤ"rep.tumor grade(Nottingham grade): Grade 1 =I; Grade 2 =II; Grade 3 =III
"β" rep. lesion size (cm): pT1= ≤ 2 cm; pT2 = 2-5 cm; pT3 = >5cm
"ɤ" rep. node positivity: pN0= 0 nodes;pN1 = 1-3 nodes;pN2 = >3 nodes.

Table 3: Frequency distribution according to ER and PR expression status

ER Score	Frequency (%)	Cumulative	PR Score	Frequency (%)	Cumulative	Interpretation
Zero	8 (3.9)		Zero	15 (7.3)		Negative
2	70(34.1)	78 (38.0)	2	45(22.0)	60 (29.3)	Negative
3	43 (20.9)		3	61 (29.8)		Positive
4	27 (13.2)		4	13 (6.3)		Positive
5	25 (12.2)	127	5	55(26.8)	145 (70.7)	Positive
6	14(6.8)	(62.0)	6	12 (5.9)		Positive
7	18(8.9)		7	4(1.9)		Positive
Total	205 (100)	205(100)	Total	205 (100)	205 (100)	

ER -Oestrogen receptor; PR - Progesterone receptor

Table 4: Frequency distribution according to Her-2/*neu* and p53 expression status

Her-2/*neu* Score	Freq. (%)	Cumulative	Interpretation	p53 Score	Freq. (%)	Cumulative	Interpretation
Zero	21 (10.2)	81	Negative	Zero	13(6.3)	49	Negative
1+	60 (29.3)	(39.5)	Negative	1+	36(17.6)	(23.9)	Negative
2+	54(26.3)	54(26.3)	Equivocal	2+	85(41.5)	156 (70.1)	Positive
3+	70(34.2)	70(34.2)	Positive	3+	71(34.6)		Positive
Total	205(100)	205(100)		Total	205(100)	205(100)	

Her-2/*neu* – human epidermal growth factor receptor-2

Table 5: Expression profile of hormone receptors, Her-2/*neu* and p53 compared to Nottingham tumor grade

Immunohistochemical markers	Nottingham grade			χ^2, *P-value*, df
	Grade I	Grade II	Grade III	
ER+ (%)	10 (7.9)	103(81.1)	14 (11.0)	
ER- (%)	3 (3.85)	53 (67.95)	22 (28.21)	10.458, *<0.01*, 2
PR+ (%)	8(5.6)	121 (83.4)	16 (11.0)	18.581, *<0.001*, 2
PR- (%)	3 (5.00)	35 (58.33)	22 (36.67)	
Her2/*neu*+ (%)	4 (5.7)	46(65.7)	20(28.6)	27.317, *<0.001*, 4
Her2/*neu*-ve (%)	31 (38.27)	42 (51.85)	8(9.88)	
Her2/*neu*-Eq (%)	11 (20.37)	28 (51.85)	15 (27.78)	
p53+ (%)	8 (5.2)	118(75.6)	30 (19.2)	13.381, *0.001*, 2
p53- (%)	9 (18.37)	38 (77.55)	2 (4.08)	

ER: oestrogenreceptor; PR: progesterone receptor; Her2/neu: human epidermal growth factor receptor 2; +: positive; -/-ve: negative; Eq.: equivocal

Fig. 1a

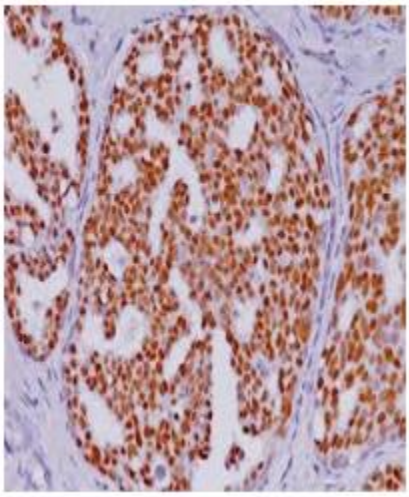

Fig. 1b

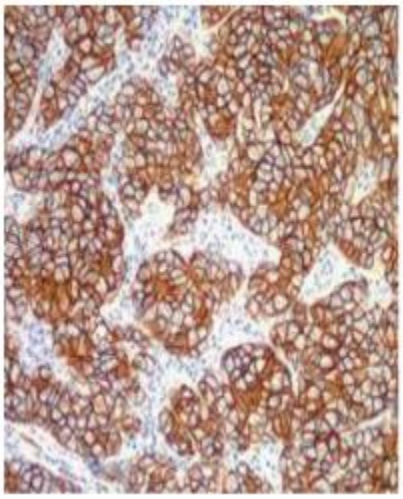

Fig. 1c

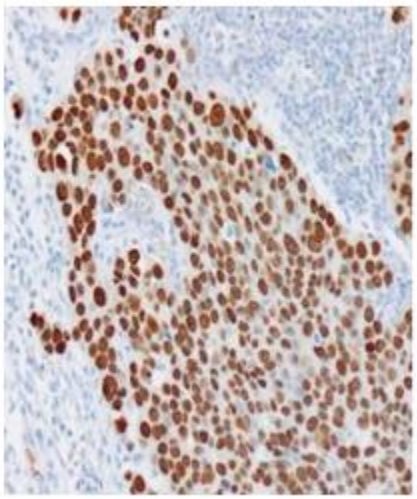

Figure 1: A: Invasive ductal carcinoma (ER-positive X40). Note that the tumor cells pick up the stain in the nucleus. The score in this case was 7. **B:** Invasive ductal carcinoma. (Her-2/neu positive x40). Note that the intensity score for this case was 3. Her-2/*neu* stains in the membrane compared to ER/PR which stains in the nucleus. **C:** Invasive ductal carcinoma (p53 positive x40). Note that the intensity score for this case is 3. p53 stains in the nucleus like ER/PR.