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CLINICAL & BASIC RESEARCH

Expression Patterns of ER, PR, HER-2/*neu* and p53 in Association with Nottingham Tumour Grade in Breast Cancer Patients

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abstract: ***Objectives:*** Recent molecular studies show that breast cancer (BC) 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 personalised treatment. However, this may be unrealistic in the resource-limited parts of the world. Thus, this study aimed to investigate the expression pattern of hormone receptors and p53 tumour suppressor using immunohistochemistry (IHC) in BC compared to the traditional tumour grade. ***Methods:*** In total, 205 cases were investigated, and the Modified Bloom-Richardson score system was adopted in grading the tumours. The tissue sections of the cases were stained with specific primary antibodies at dilutions of 1:60 for oestrogen receptors (ER) and progesterone receptors (PR), 1:350 for the human epidermal growth factor (HER-2/*neu*) and 1:50 for p53. ***Results:*** Invasive ductal carcinoma of no-specific type (n = 190, 92.7%) was predominant and grade II tumour (n = 146, 71.2%) was the most frequent. Hormone receptors ER (n = 127) and PR (n = 145) had 62.0% and 70.7% positive cases, respectively; 34.1% (n = 70) were positive for HER-2/*neu*, while 76.1% (n = 156) were positive for p53. Significant 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) were observed. ***Conclusion:*** Nottingham grade had 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; Heterogeneity; Hormone Receptor; Oncoprotein p53.

Advances in Knowledge

- *-Grade II tumours displayed higher levels of oestrogen receptor (ER) and progesterone receptor (PR) expression than grade III tumours, indicating that as the disease progresses, the proportion of cells expressing ER and/or PR steadily declines.*
- *Similarly, higher hormone receptor (HR) positivity was observed in the current study than in many black populations, including Guinea, Ghana, South Africa and Mali, emphasising potentially identifiable intra-racial factors influencing the diverse variation.*
- *Some patients had higher grades in the human epidermal growth factor (HER-2/neu)+ expression group than in the HER-2/neu– expression group while having lower grades in ER+ and PR+ expression groups than the ER– and PR– group of the highest grade III.*
- *This study 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+ tumours, indicating that these cancer types are responsive to hormone treatment, have a better prognosis and are less aggressive. This observation contrasts with the prevailing belief that the black population tends to exhibit aggressive breast cancer presentation.*
- *The proportion of TNBC patients was relatively low, implying that hormone or targeted therapies focusing on HER-2 would benefit the majority of cancer patients.*

Application to Patient Care

- *The cancer phenotype can exhibit location-dependent variations due to several factors, including genetic predisposition, lifestyle and environmental influences.*
- *Further, it was observed that 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 tumour grade, can help guide personalised treatment decisions, optimise therapy selection and potentially improve patient outcomes.*

Cancer continues to be one of the deadliest non-communicable diseases worldwide.[1] Although the literature shows that breast cancer (BC) is more common in developed countries, a recent GLOBOCAN estimate shows that Africa constitutes a nerve-racking proportion of BC deaths,

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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. The histological method is still commonly and exclusively used in many African countries, especially in low-resource settings.[3]

In this new genomic era, molecular markers are gaining wide acceptance as sensitive and inclusive methods to understand the behaviour of advanced cancers. Specifically, hormone receptors (HR) 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 primarily used.[4] Each of these biomarkers is important in diagnosing BC and may sometimes correlate with other diagnostic indicators. The overexpression of HER-2/*neu* has been linked to a higher histological grade, increased tumour size, the number of affected lymph nodes, p53 mutation and lower oestrogen receptor (ER) expression (or even ER expression in some cases).[5] Similarly, ER and progesterone receptor (PR) patterns have been linked to BC grade, thereby potentially influencing treatment options.[6,7] Furthermore, a mutation in the p53 gene, which is a tumour suppressor gene, represents a genetic predisposition to cancers and has been associated with tumour aggressiveness, making them a possible indicator of histological grade.[8,9]

Meanwhile, histological grade enables a description of a tumour's level of aggressiveness and is regarded as a forerunner for the morphological evaluation of tumour's biological characteristics.[10] According to a study on gene expression, histological grade reveals information about the molecular makeup of BC in addition to tumour size or lymph node involvement.[11] Furthermore, evidence from genome-wide microarray-based expression profiling elucidates many characteristics of tumour biology in BC, adding to the evidence that the biological features revealed by histological grade are critical in determining tumour behaviour.[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 tumour grading and the more contemporary immunohistochemistry (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, the frequency and patterns of expression of some clinically significant molecular markers in patients with BC were investigated. Furthermore, the link between the biomarkers' expression patterns and histological tumour grade was explored to determine their role in disease diagnosis.

Methods

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

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

Data about the age, histological grade, nuclear grade, tumour size and lymph node involvement were extracted from the patients' records.

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

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, Agilent Technologies, Santa Clara, California, USA), PR (Dako), HER-2/*neu* (ERBB2; Dako) and p53 (Do-7; Santa Cruz Biotechnology Inc., Dallas, Texas, USA) at the Breast Cancer Laboratory Medical Genetic and Bioethics Research Unit, Institute for Advanced Medical Research and Training, University College Hospital, Ibadan, Nigeria. 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 1 hour). Negative and positive controls were performed by including the control tissues specified by the antibody vendors.

The scoring was performed using the modified immunohistochemical score ('Quickscore'), a modified

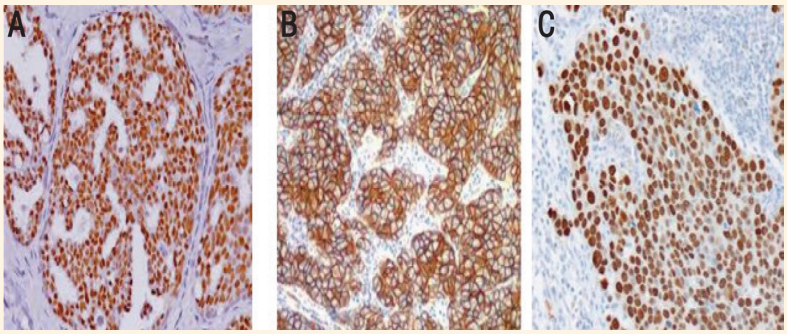

Figure 1: A: Oestrogen receptor (ER)-positive stain at × 40 magnification showing invasive ductal carcinoma (note: that the tumour cells pick up the stain in the nucleus, and the score in this case was 7). **B:** Her-2/*neu* positive stain at × 40 magnification showing invasive ductal carcinoma (note: the intensity score for this case was 3 and Her-2/*neu* stains in the membrane compared to ER/progesterone receptor [PR] which stains in the nucleus). **C:** p53 positive stain at × 40 magnification showing invasive ductal carcinoma (note: the intensity score for this case is 3, and p53 stains in the nucleus like ER/PR).

semi-quantitative assessment method by Allred *et al*.[13] Nuclear staining intensity was scored from 0 to 3+ in combination with the proportion of cells involved to achieve 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 and 7 = good (75%) chance of benefit.

For HER-2/*neu* expression, the only membrane staining pattern was scored from 0 to 3+, where 0/1+ indicates negative, 2+ indicates equivocal, and 3+ indicates 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 tumour cells or no staining detected; negative (1+ score) = membrane staining detected in >10% of the tumour cells or faint staining detected, and 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 tumour cells; positive (3+ score) = a strong complete membrane staining was detected in >10% of the tumour cells. The molecular classification was based on the positivity and negativity of ER, PR, and HER-2/*neu* [Figure 1].

Table 1: Scoring guideline ('Quickscore') for oestrogen receptors and progesterone receptors

Proportion score	Observation	Intensity score	Observation
0	Zero staining	0	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.

For p53 expression, the nuclear staining pattern was scored from 0 to 3+, where 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 of staining was used to determine whether the p53 protein was overexpressed or not. The numbers 0 and 1+ indicated negative staining, while the numbers 2+ and 3+ indicated positive staining [Figure 1C]. The p53 protein was considered negative if it was not overexpressed or mutated.

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

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

Table 2: Frequency distribution of tumour grade, size and lymph node involvement in female breast cancers (N = 205)

Tumour index	n (%)
Tumour grade*	
I (low)	16 (7.8)
II (intermediate)	146 (71.2)
III (high)	43 (21.0)
Tumour size†	
pT1	18 (8.8)
pT2	106 (51.7)
pT3	81 (39.5)
Lymph node status‡	
pN0	156 (76.1)
pN1	46 (22.4)
pN2	3 (1.5)

**Tumour grade (Nottingham grade): Grade 1 = I; Grade 2 =II; Grade 3 =III. †Lesion size in cm: pT1 = ≤2 cm; pT2 = 2–5 cm; pT3 = >5cm. ‡Node positivity: pN0 = 0 nodes; pN1 = 1–3 nodes; pN2 = >3 nodes.*

Results

A total of 205 cases were investigated for IHC markers ER, PR, HER-2/*neu* and p53 immunomarkers. The age range was 21–87 years (mean = 49.30 years). The peak age of this incidence was 50–59 years. By laterality, the records showed that BC occurred at nearly the same rate between the left (n = 103, 50.2%) and the right (n = 102, 49.8%) breast sides among those with complete records.

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

Using Nottingham modification of the Bloom–Richardson system, the frequency distribution by tumour grade was recorded [Table 2]. All the cases had specified tumour sizes ranging between 1 and 22 cm in the widest diameter (mean = 5.8 cm) [Table 2]. 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 Nottingham Prognostic Index (NPI) traditionally involves a combination of the assessments of nodal status, tumour 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 to ≤3.4; NPI-III (moderate) >3.4 to ≤5.4; and NPI-IV (poor) >5.4.16 The data in the present study revealed that out of the 205 cases, 63 (30.7%) cases showed a good prognosis, 100 (48.8%) showed a moderate prognosis and 42 (20.5%) showed a poor prognosis.

A total of 205 BC cases were processed and stained for ER, PR, HER-2/*neu* antigen and p53 positivity and immunostained for ER and PR. Out of these, 127 (62.0%) cases were ER+, 78 (38.0%) cases were ER–, 145 (70.7%) were PR+ and 60 (29.3%) were PR– [Table 3 and Figure 1]. A total of 70 (34.1%) cases were HER-2/*neu*+, 81 (39.5%) were HER-2/*neu*– and 54 (26.3%) were equivocal. For the equivocal result, the stains were not furthered with fluorescent *in situ* hybridisation (FISH) due to limited funding but considered HER-2/*neu*– [Table 4 and Figure 1B]. A total of 156 (76.1%) cases were p53+, while 49 (23.9%) cases were p53– [Table 4 and Figure 1C].

Associations between the expression profile of HR (ER and PR), HER-2/*neu* and p53 were observed compared to the Nottingham tumour grade. Furthermore, the pattern of expression in ER (positivity) showed a significant difference (P <0.01) compared to the distribution of patients according to tumour grades in the same way as PR positivity (P

Table 3: Frequency distribution according to oestrogen receptor and progesterone receptor expression status (N = 205)

ER score	n (%)	Total	PR score	n (%)	Total	Interpretation
Zero	8 (3.9)	78 (38.0)	Zero	15 (7.3)	60 (29.3)	Negative
2	70 (34.1)		2	45 (22.0)		Negative
3	43 (21.0)		3	61 (29.8)		Positive
4	27 (13.2)		4	13 (6.3)		Positive
5	25 (12.2)	127 (62.0)	5	55 (26.8)	145 (70.7)	Positive
6	14 (6.8)		6	12 (5.9)		Positive
7	18 (8.8)		7	4 (2.0)		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 (N = 205)

HER-2/ neuscore	n (%)	Cumulative	Interpretation	p53 score	n (%)	Cumulative	Interpretation
Zero	21 (10.2)	81 (39.5)	Negative	Zero	13 (6.3)	49 (23.9)	Negative
1+	60 (29.3)		Negative	1+	36 (17.6)		Negative
2+	54 (26.3)	54 (26.3)	Equivocal	2+	85 (41.5)	156 (76.1)	Positive
3+	70 (34.1)	70 (34.1)	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.

<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 tumour grade pattern. Additionally, an association (P = 0.001) between the p53 expression pattern and the Nottingham tumour grade pattern was observed.

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

The number of ER/PR positive, HER-2/*neu* negative cases was 110 (53.7%). This subtype was characterised by the presence of ER and PR but the absence of Her-2 overexpression through ER+/PR+, Her-2–; ER–/PR+, Her-2– and ER+/PR–, Her-2–.

The number of ER/PR positive, HER-2/*neu* positive cases was 48 (23.4%). This subtype was defined by the presence of both ER and PR, as well as Her-2 overexpression through ER+/PR+, Her-2+: ER–/PR+, Her-2+ and ER+/PR–, Her-2+.

The number of ER/PR negative, HER-2/*neu* positive cases was 22 (10.7%). This subtype was identified by the absence of ER and PR, but the presence of Her-2 overexpression through ER–/PR–, Her-2+.

The number of triple-negative cases was 25 (12.2%). This subtype was specified by the absence of ER and PR, as well as Her-2 overexpression through ER–/PR–, Her-2– and ER–/PR–, Her-2–.

Discussion

This study retrospectively investigated 205 BC cases in western Nigeria for ER, PR, HER-2/*neu* and p53 expression profiles in terms of pattern and frequency. Furthermore, it explored the expression patterns of these biomarkers in connection with tumour's aggressiveness by using the conventional Nottingham grade. The molecular characteristics of the tumour showed that ER and PR were positive in 62.0% and 70.7% of the total recorded cases, respectively. There were associations between ER and PR's expression patterns and the tumour grades' frequency. This is following the report on Polish women that showed an association between tumour grades and HR positivity.[17] The present study showed that grade II tumours had a higher ER and PR positive frequency than grade III. Meanwhile, a previous report indicated that the number of cells expressing ER and/or PR gradually decreases with disease progression.[18] This

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

Immunohistochemical markers	Nottingham grade, n (%)			χ^2	*P* value	df
	Grade I	Grade II	Grade III			
ER+	10 (7.9)	103 (81.1)	14 (11.0)	10.458	<0.01	2
ER–	3 (3.8)	53 (67.9)	22 (28.2)			
PR+	8 (5.6)	121 (83.4)	16 (11.0)	18.581	<0.001	2
PR–	3 (5.0)	35 (58.3)	22 (36.7)			
HER2/*neu*+	4 (5.7)	46 (65.7)	20 (28.6)			
HER2/*neu*–	31 (38.3)	42 (51.9)	8 (9.9)	27.317	<0.001	4
HER2/*neu*-Eq	11 (20.4)	28 (51.9)	15 (27.8)			
p53+	8 (5.1)	118 (75.6)	30 (19.2)	13.381	0.001	2
p53–	9 (18.4)	38 (77.6)	2 (4.1)			

ER = oestrogen receptor; PR = progesterone receptor; HER2/neu = human epidermal growth factor receptor 2; Eq = equivocal.

was substantiated by Badowska-Kozakiewicz *et al.* who showed an inverse correlation between ER expression and the size of the primary tumour.[17] Specifically, in addition to positively predicting therapeutic outcomes, ERα is believed to inhibit epithelial-mesenchymal transition by promoting epithelial phenotype and preventing tumour invasion in breast cancer.[19]

Moreover, higher HR positivity was observed than in many African populations, including Guinea, Ghana, South Africa and Mali.[20–23] 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 this study showed higher HR positivity compared to a study of a similar population in Nigeria, where a multicentric study involving 507 patients was carried out.[24] Conversely, the findings of this study are in line with reports involving BC patients in Western countries and the Saudi population, where high HR was also documented.[6,7] Potemski *et al.* reported related results, which revealed that the higher the level of receptor expression, the lesser the mortality.[25] In line with their observations, the current study also showed that the majority of the incident cases had a moderate prognosis, with high HR positivity and lower tumour grades, indicating a possible association between HR expression and tumour grade.

With regard to the HER-2/*neu* expression pattern in this study, some (39.5%) cases were negative; there were more negative than positive (34.1%) outcomes, 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 the present study, Arafah 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.[7] Furthermore, Aman *et al.* associated overexpression of HER-2/*neu* with higher Nottingham grade in an Ivorian population.[26] Again, in line 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 the current study were infiltrating ductal carcinoma, which is consistent with Ding *et al.*'s study, the present analysis also showed a strong association between histological grading and the pattern of expression of HER-2/*neu*.[9] However, the observations in this study indicated that HER-2/*neu* overexpression is linked to the aggressive forms of BC, as previously reported by Arteaga *et al.*[27]

To better understand the therapeutic benefits for the patients, the current study classified the patients based on histological phenotypes of the HR and HER-2/*neu* expression patterns. In line with Gago *et al.*'s report, the majority of the BC patients in this study co-expressed HR+ with HER-2/*neu*– rather than Her-2+ tumours, indicating that the cancer cells are responsive to hormones such as oestrogen and progesterone, have better prognosis and also prevent tumour aggressiveness.[28] On the other hand, among the Her-2+ category, a smaller number of ER/PR-HER-2/*neu*+ was observed representing BC cases where both the ER and PR are negative, while the HER-2/*neu* is overexpressed. This subtype is commonly known as HR-negative, HER-2/*neu*-positive BC. 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 BC (TNBC) is a vastly diverse group of tumours, which represents 15–20% of all breast cancer cases.[29] The proportion of the TNBC in this study was relatively small, suggesting an advantage against the studied population. Meanwhile, TNBC is the most difficult to treat among all BC phenotypes because the common hormonal therapy used for the majority of BC subtypes was treatment-refractory for TNBC. On the other hand, TNBC is often treated in its early stages with surgery, radiation and chemotherapy.

Furthermore, in the present study, most of the investigated cases (70.1%) were p53 positive, and there was a strong association between the p53 expression pattern and the Nottingham tumour grade. Consistent with other studies, the findings of this study imply that the p53 positivity may have a connection with tumour grade in terms of the frequency of the incident cases.[5,30] In this study, patients in the p53+ expression group were classified histologically as higher grades than those in the p53- expression group similar to the previous report, which corresponds to the HER-2/*neu* expression pattern in this investigation.[24] Earlier, Shokouh *et al.* demonstrated that p53 expression had a significant association with the grade of BC.[5] 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 tumours 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 tumour's high mitotic index.[8]

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

Conclusion

Expression patterns of PR, ER, HER-2/*neu* and p53 were influenced by the tumour grade (i.e. level of aggressiveness). In other words, an association between the tumour grade and expressions of PR, ER, HER-2/*neu* and p53 was observed, suggesting that the Nottingham grade remains relevant as a reliable prognostic marker for BC.

AUTHORS' CONTRIBUTION

KAO, WAO and MAO were involved in conceptualisation and design of the study. WAO, MAO, LAY and RTK collected the data, while KAA, SOI and RTK analysed and interpreted the results. KAA, SOI, IAL, IOB and SAA did the literature search. WAO carried out clinical studies. KAA drafted the manuscript. Then, KAA, SAA and SOI revised the manuscript. All authors approved the final version of the manuscript.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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