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Her-2/neu overexpression in breast cancers in patients of West African extraction seen in Lagos state University Teaching hospital, Nigeria

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ABSTRACT

Introduction: Her-2/neu is one of the most important molecular markers of breast cancer. Over-expression of Her-2/neu as evaluated by immunohistochemistry is necessary in the management of breast cancers. This study was performed to determine the proportion of expression of the biomarker amongst breast cancer patients who presented in our 'one-stop breast cancer unit' using automated immunohistochemistry.

Materials and methods: Automated immunohistochemical analysis of 107 newly diagnosed breast cancer patients was done for expression of Her-2/neu, between 1st April, 2016 and 30th September, 2018. The data was analyzed using SPSS version 25 for windows and Microsoft excel, 2013.

Results: Her-2/neu was overexpressed in 30.1% patients, and it shows no correlation with age and histological grade of the disease.

Conclusion: There is overexpression of Her-2/neu in our local setting, and this observation is very relevant to any therapeutic decisions and management of these patients.

Introduction

Breast cancer is the commonest cancer in women worldwide, including Nigeria [1]. The cancer is also associated with a high mortality, especially when the patient presents at a late stage and has a high grade disease [2]. Breast cancer is characterized by genetic heterogeneity, thus making treatment plans for patients different in view of the fact that tumours with same histopathological characteristics show different clinical response and aggressiveness [2]. Improvement in breast cancer diagnosis and treatment has revolutionized the outcome of the disease [3]. Scientific knowledge of the existence of Her-2/neu receptors on breast cancer cells has provided room for use of targeted therapy against breast cancers [4].

Southern blot testing, polymerase chain reaction amplification, fluorescence in-situ hybridization assays, chromogenic in-situ hybridization and immunohistochemistry techniques are designed to detect Her-2/neu gene amplification [5]. The American Society of Clinical Oncology (ASCO) and College of American Pathologists (CAP) issued a joint updated guideline on human epidermal growth factor receptor 2 testing in breast cancer [6,7]. The document reflects an extensive medical literature review and recommendations for testing Her-2/neu positivity, interpreting the results, and determining Her-2/neu targeted therapies. Originally released in 2007, the guideline was updated to strengthen and clarify prior recommendations based on evidence that has surfaced in the ensuing testing for Her-2/neu. The guideline recommended testing for Her-2/neu either by immunohistochemistry or in-situ hybridization [6,7].

Applying immunohistochemical methods for the assessment of sections of breast tumours, using antibodies to the Her-2/neu protein, allows investigation of tumours for overexpression of the protein, and relating protein expression to tumour prognosis [8].

Her-2/neu overexpression is amplified in 20–25% of primary breast cancers and has been associated with poor prognosis [9]. The association between Her-2/neu amplification and poor prognosis was first determined in 1987 by Slamon and co-workers [9].

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Amplification of Her-2/neu gene was shown to strongly correlate with time of relapse and overall survival. Her-2/neu status is also important for selecting those with metastasis for therapy with trastuzumab (Herceptin) [10].

Pre-analytic variables should be optimized in getting the best immunohistochemistry(IHC) result [6,11]. Good fixation of specimens in 10% neutral buffered formalin in a ratio of tissue to formalin of 1:20, fixation of tissue for at least 6hrs and maximum 72hrs, short cold ischaemic time, preferably less than 1hr are all measures to get satisfactory Her-2/neu results [11]. Good analytical procedures, including choice of right antibodies, controls as well as interpretation by well-trained Pathologists are important in getting good results [6,11].

Automated immunohistochemistry allows for reproducibility, reduces the challenges of staff shortages, offers process for monitoring for errors with alarms for events which are necessary in quality control management [12].

Material and methods

This is a prospective study that was carried out in the department of Pathology and Forensic Medicine, LASUTH, Ikeja, Lagos state, Nigeria between 1st April, 2016 and 30th September, 2018. Lagos state is the commercial nerve centre and the most populous state in Nigeria with approximately twenty million inhabitants. The state attracts people from all parts of the country, as well as foreign nationals thus, adding to the large population and diversity. The department of Pathology and Forensic Medicine receives histopathological samples from the 'one-stop breast cancer clinic' within the department, which adopts a multidisciplinary approach to the management of breast cancers. The multidisciplinary team is composed of Pathologists, Surgeons, Oncologists, Radiotherapists and Oncology nursing assistant. The pre-analytical factors are well controlled to ensure optimal result.

The authors set out to find out the proportion of expression of Her-2/neu in breast cancers in Lagos State University Teaching Hospital. Other objectives include the determination of any correlation between the age of patients, histological grade of the breast carcinoma and Her-2/neu expression.

All patients who were newly diagnosed as breast cancer patients, and had core needle biopsies done in our 'one-stop breast clinic' were included in the study. Cases from peripheral medical centers, private and general hospitals within and outside the state were excluded from the study. Breast samples from patients on chemotherapy and/or radiotherapy were also exempted from the study.

The recruitment of the patients was through the clinic; they comprised those who had palpable breast lesions. At least two passes of core needle biopsy of the breast were done and the tissues immediately fixed in 10% neutral buffered formalin. Grossing and processing of these tissues were within 24 h and not less than 8 h. The formalin fixed paraffin embedded blocks were sectioned at 4μ and placed on a commercially prepared charged slide. Control blocks were also cut and placed on the same slide. The slides were loaded onto the Ventana Benchmark GX machine (SN 815224; REF 750-850) used for auto immunostaining in our facility. The immunostaining protocol used for the runs is as stated below, and the machine has been programmed accordingly:

a. The sections were deparaffinized.
b. Antigen retrieval was done using the slide heater in Ventana Benchmark.
c. The incubation time was for 60 min at 100 °C.
d. The primary antibody used was Her-2/neu, 4B5.
e. The incubation time was for 32 min at 42 °C.
f. Full automated staining was done within the Benchmark.
g. Consumables were supplied by Roche and bulk fluid usage include: cell conditioner 1, lot number (131795-01; 231342-01), EZ Prep (132 322-01; 245 650-01), liquid cover slipping (128 746-01, 128-746-01, 245 657-01) and reaction buffer (138 025-01, 193 461-01).
h. The slides were retrieved from the machine, washed gently in soapy water after a run of 2 h and 30 min. The slides were then dried and cover-slipped with DPX mountant.

The slides were subsequently reviewed by trained and experienced pathologists in immunohistochemistry techniques.

The scoring was according to the 2013/2018 updated ASCO/CAP recommendation for Her-2/neu scoring [6,7]. The Staining Pattern Score for Her-2/neu Staining Assessment are as below:

No membrane staining is observed = 0 negative

Faint, partial staining of the membrane in any proportion of the cancer cells = 1 + Negative.

Weak to moderate complete staining of the membrane, greater than 10% of cancer cells = 2 + Equivocal.

Strong, complete staining of the membrane greater than 10% of cancer cells = 3 + Positive.

Statistical significance was set at $P < 0.05$. Data was analysed using Chi Square test. Statistical analysis was performed using the Statistical Package for Social Science (SPSS) version 25 for windows and Microsoft excel 2013.

Results

There were 107 breast carcinoma specimens from 3 (2.8%) males and 104 (97.2%) females with age range of 26–88 years. The total population of breast cases seen was 840. The mean age was 52 ± 13 years. Majority of the patients were within the age group of 40–59 (54.0%), 16.8% were aged 60–69 years while 14.0% were aged 30–39 years (Fig. 1). Most of the patients (71.0%) presented with a grade III tumour while the rest (29.0%) showed a grade II pattern. Most of the samples stained negative for Her-2/neu (Fig. 2); the morphological appearances of the staining pattern are shown in Fig. 3.

The percentage Her-2^{+} increases from 0 for <30 years to 44.4% for 60–69 years and a slight decrease to 25% for 70 years and above. On the other hand, Her-2/neu $^{-ve}$ decreased from 100% for <30 years to 56.6% for 60–69 years with an increase to 75.0% for 70 years and above. Her-2/neu equivocal was seen in 20.0% of patients within the 30–39 years' age group, 3.3% for those within 40–49 years, and absent in other age groups (Table 1). Her-2 positive was 35.5% in patients with histological grade II and 28.9% in patients with histological grade III. Her-2 negative was 61.3% in patients with histological grade II and 67.1% in those with grade III. Her-2 equivocal was 3.2% and 3.9% among patients with grades II and III

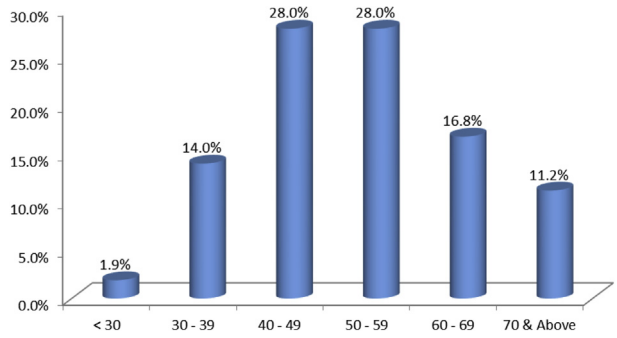

Fig. 1. Age distribution of the patients.

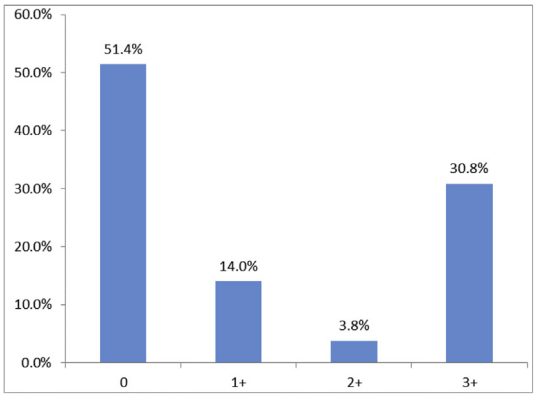

Fig. 2. Her-2/neu positivity.

respectively (Table 2). There were 30.8% Her-2/neu positivity, 65.4% negativity and 3.8% equivocal with 54.2%, 3.6% and 7.5% distributions for positivity, negativity and equivocal respectively (Table 3).

Discussion

The effective management of patients with breast cancer needs knowledge of the hormone receptor status. The responsiveness of these tumours to hormone therapy is very vital to breast management and the patient's survival.

The mean age for the diagnosis of breast cancer was 52 ± 13 years. Age was found not to be correlated with Her-2/neu expression in breast cancer ($p > 0.05$). This is in agreement with the study conducted by Rosen and co-workers [13] in which age was said not to be related to Her-2/neu positivity in breast cancer. It is interesting to note that all patients aged less than 30 years in our study had Her-2/neu negativity. The authors hope to further critically study the behavior of breast cancer in this group of patients in a future study.

The Her-2/neu positivity was found to be 30.8% in this study; reports from other parts of Nigeria have shown values of 22.0%, 11.4%, 20.8% from Maiduguri (North-eastern Nigeria) [14], Nnewi

Table 2
Her-2/neu versus histological grade ($p = 0.079$).

Marker	Histological Grade (%)		Total
	II	III	
Positive	11 (35.5%)	22 (28.9%)	33 (30.8%)
Negative	19 (61.3%)	51 (67.1%)	70 (65.4%)
Equivocal	1 (3.2%)	3 (3.9%)	4 (3.8%)
Total	31 (100.0%)	76 (100.0%)	107 (100.0%)

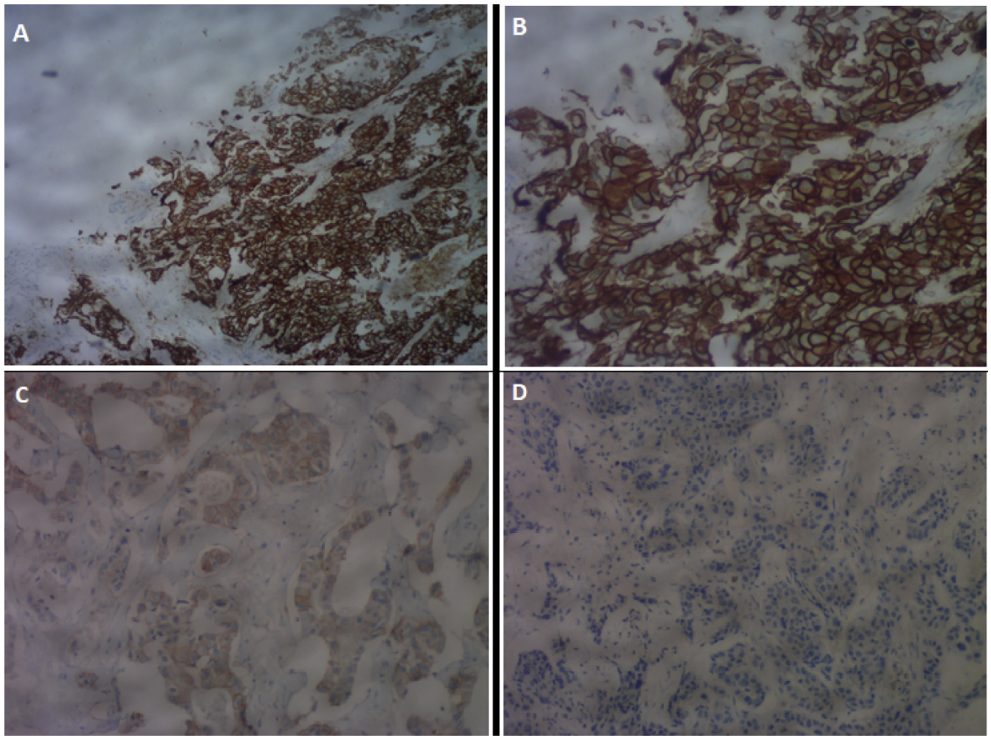

Fig. 3. A: Her-2/neu positive (3+) of a Grade III Invasive ductal carcinoma (x 40), **B:** Her-2/neu positive (3+) of a Grade III Invasive ductal carcinoma (x 100), **C:** Her-2/neu positive (2+) Equivocal of a Grade III Invasive ductal carcinoma (x 100), **D:** Her-2/neu negative of a Grade III Invasive ductal carcinoma (x 100).

Table 1
Her-2/neu status versus Age group ($p = 0.078$).

HER-2/NEU	Age category (%)					
	<30	30–39	40–49	50–59	60–69	70+
Positive	-	3 (20.0%)	8 (26.7%)	11 (36.7%)	8 (44.4%)	3 (25.0%)
Negative	2 (100.0%)	9 (60.0%)	21 (70.0%)	19 (63.3%)	10 (56.6%)	9 (75.0%)
Equivocal	-	3 (20.0%)	1(3.3%)	-	-	-
Total	2 (100.0%)	15 (100.0%)	30 (100.0%)	30(100.0%)	18 (100.0%)	12 (100.0%)

Table 3
Positivity and Percentage distribution of Her-2/neu marker.

	Her-2/neu		
	Positive	Negative	Equivocal
Number (%)	33 (30.8%)	70 (65.4%)	4 (3.8%)
Distribution (%)	54.24%	3.57%	7.50%

(South-eastern Nigeria) [15] and Benin (Mid-western Nigeria) [16] respectively. The present authors believe that variations in protocols, pre-analytical variables, and the use of an automated equipment in our facility most probably account for the low values reported from other parts of the country. The one-stop-diagnostic breast clinic at the authors' facility not only minimize pre-analytic variables, it also benefits the patient. The latter is offered a quick diagnosis and initiation of treatment. The patient is not lost in the course of delays in getting the samples taken, diagnosis made, and treatment initiated.

It is noteworthy that Seshie and colleagues reported 25.5% Her-2/neu positivity in a retrospective analysis of breast cancer subtype done in Korle Bu Teaching Hospital, Ghana, West Africa [17]. Studies by Yau and co-workers [18] reported Her-2/neu expression of 21.0% in breast cancer cases seen in Hong Kong while Mahyari and colleagues [19] observed 38% among Iranian woman with early stage breast cancer. A comparative multicenter study that ensures a uniform protocol and minimal analytical variations would be necessary to explain the variations observed. The strict inclusion and exclusion criteria used in this study are recommended by the authors for such a study.

Several authors have found a correlation between the grade of tumours and Her-2/neu positivity. Rasheed and colleagues found that poorly differentiated invasive ductal carcinoma have high Her-2/neu positivity [8]. Sun et al. found that poorly differentiated clusters in invasive breast cancer is also associated with Her-2/neu overexpression [20]. This outcome cannot be effectively compared with our study in view of the fact that most of the tumours fall into grade II and III. Comparative study of larger sample size in Africans or African Americans will be necessary to establish this association.

Conclusion

Her-2/neu is an important biomarker in the evaluation of breast cancer. The knowledge of its positivity is vital to proper management of breast cancers. In our local setting, Her-2/neu positivity was 30.8%, meaning that 3–4 patients out of 10 with breast cancers overexpress this antigen. It is now mandatory that all breast cancer patients in this environment be tested for the expression of this antigen. Our patients have started benefitting from this study. There is an increased awareness among clinicians and patients, and the management of the latter has been positively impacted upon. We hope that our patients will present earlier in the future for diagnosis and treatment, and with a lower tumour grade. The one-stop diagnostic breast clinic is expected to continue in order to save time between diagnosis and treatment.

Conflict of interest statement

The authors have no conflict of interest. This study was not funded by any individual or organization.

Declarations of interest

None.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ejso.2019.06.037.

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