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Prognostic Implications of ER, PR and HER2/Neu Protein Expression in a Cohort of Breast Carcinoma

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Abstract: ER, PR and HER 2/neu receptor studies are known to be prognostic and predictive biomarkers of breast carcinoma. Hence the status of ER, PR and HER 2/neu receptors are routinely assessed in breast carcinoma by immunohistochemistry using paraffin embedded tissue blocks. The reason for assessing the receptor status is to decide on the best treatment regime for breast cancer patients. Ki 67 which is a biomarker of cellular proliferation is also assessed to guide the oncologist by determining the proliferative capacity of the tumour. A descriptive cross-sectional study conducted during January 2016 to December 2018 in three specialized surgical centres. Study sample included 92 patients with histologically confirmed breast carcinoma. ER, PR, HER2/neu receptor status and Ki 67 proliferative index were assessed to determine the prognostic implications of breast carcinoma. Mean age at presentation was 53.99 years and the most common histological type was invasive ductal carcinoma (84.78%). In the cohort of 92 patients with breast carcinoma 73.91% were ER positive, 58.69% were PR positive, and 11.95% were HER2/neu positive. Lymph nodal involvement was seen in 31.52% of the patients. There was no statistically significant association with HER2/neu status and nodal involvement ($p = 0.629$). Distant metastasis was seen in 4.35% cases. The association with HER2/neu status and distant metastasis was not statistically significant ($p = 0.085$). ER status showed a significant negative correlation with HER2 status (rho = -0.634, $p < 0.0001$). PR status showed a significant negative correlation with HER2 status (rho = -0.834, $p < 0.0001$). Ki67 index showed a significant positive correlation with HER2 status (rho = 0.248, $p = 0.017$).

Keywords: Breast Carcinoma, HER 2/Neu Protein, Hormone Receptors

1. Introduction

Breast carcinoma is the most common malignancy in females worldwide [1]. It is the leading cause of death in women due to cancer [2]. In most parts of the world there is a rise in the detection rate of breast carcinoma due to the availability of mammography and increased awareness among different communities across the world [3]. Mammography enables to identify extremely small lesions which are few millimeters in diameter. In order to ensure uniformity in interpretation the mammographic findings are now universally reported using the Breast Imaging Reporting and Data System [4, 5].

The identification of receptors in breast cancer cells has revolutionized the management of patients by providing prognostic and predictive information for the oncologists [6]. The role of the pathologist is to correctly assess the receptor status of tumour tissue by a histological technique such as immunohistochemistry. The main advantage of immunohistochemistry is that it does not require fresh tissue for the procedure. Receptor studies that are routinely done for breast carcinoma include oestrogen receptor (ER), progesterone receptor (PR) and human epidermal growth factor receptor-2 (HER2/neu). The rationale of doing receptor studies is to decide on the best treatment regime for breast

cancer patients. ER and PR are nuclear receptors that are responsive to hormone signals and promote cell growth. Allred scoring system has been used to assess the ER and PR status of the tumour. Score 0-5 is given to assess the proportion of stained tumour cells and score 0-3 is given to determine the intensity of the staining reaction. Final Allred score is the sum of the proportion score and intensity score [7].

Proportion score (PS)

Score	% of stained nuclei
0	no staining of nuclei
1	< 1% of cells show nuclear staining
2	1-10% of cells show nuclear staining
3	11-33% of cells show nuclear staining
4	34-66% of cells show nuclear staining
5	67-100% of cells show nuclear staining

Intensity score (IS)

Score	Intensity of staining
0	no staining
1	weak nuclear staining
2	moderate nuclear staining
3	strong nuclear staining

HER 2/neu proteins are transmembrane tyrosine kinase receptors found in normal breast tissue. HER 2/neu receptors control growth, division and repair of breast tissue under normal physiological conditions. In certain breast cancers HER 2/neu gene is amplified and a as a result HER 2/neu receptor will be over expressed. This will lead to uncontrolled proliferation of HER 2/neu positive breast cancers. HER 2/neu overexpression is associated with an aggressive biological behavior [8].

Table 1. *Scoring of Human epidermal growth factor receptor (HER2/neu).*

Staining pattern	Score	HER 2/neu status
No membrane staining	0	negative
Weak, incomplete, heterogenous membrane staining in > 10% tumour cells	1+	negative
Weak to moderate, complete membrane staining in > 10% tumour cells	2+	weakly positive/equivocal
Strong, complete membrane staining in > 10% of tumour cells	3+	strongly positive

Ki 67 is a cellular marker of proliferation. It is a nuclear protein encoded by the gene MK167. Ki 67 can be assessed by immunohistochemistry and is a reliable biomarker to determine the proliferative status of the tumour. Ki 67 is expressed as a percentage of tumour cells that showed nuclear staining and the counting is done in mitotically active areas of the tumour [9]. Ki-67 staining is detected by using the MIB-1 antibody.

HER 2/neu is overexpressed in approximately 15 – 30% of breast cancers [10] and is associated with shorter disease-free interval. HER 2/neu amplification also correlates with nodal metastasis and absence of ER and PR receptors [11]. There is evidence to support that HER2/neu amplification occurs early in human breast carcinogenesis. HER2/neu positive breast cancers are resistant to hormonal treatment but show increased sensitivity to cytotoxic chemotherapeutic agents. HER2/neu positive breast cancers show an increased tendency for hematogenous spread. There is evidence to support that anti-HER2 antibodies (Herceptin) can increase the disease-free interval in HER2 positive breast cancer patients [12].

Objectives:

To assess the frequency of ER, PR and HER 2/neu protein expression in a cohort of histologically confirmed breast carcinoma and determine the prognostic implications.

2. Methodology

Descriptive cross- sectional study was conducted during January 2016 to December 2018 in three specialized surgical centers of the Colombo district in Sri Lanka. Multicenter study included 92 patients with breast carcinoma. Resected surgical specimens of the patients that were adequately fixed in 10% buffered formalin were sectioned, and representative samples were chosen to be placed in tissue cassettes for overnight processing. Following tissue processing samples were embedded in paraffin wax. Paraffin embedded tissue blocks were cut in to 4 µm thick sections and stained with haematoxylin & eosin (H & E). Tumour characteristics were identified by the microscopic examination of H & E stained tumour tissue. ER, PR, HER 2/neu receptors and Ki 67 proliferative index were assessed by immunohistochemical staining of paraffin embedded tumour tissue (Dako REAL EnVision Detection System, Peroxidase/DAB). Primary antibodies that were used are monoclonal mouse anti-human oestrogen receptor α, clone 1D5 and monoclonal mouse anti-human progesterone receptor, clone PgR636. For the detection of Ki 67 monoclonal mouse anti-human Ki 67 antigen, clone MIB-1, 0.2 ml vial was used. Polyclonal Rabbit anti-human c-erbB-2 oncoprotein (Dako), LOT 20042564, 1:600 dilution, was used for the assessment HER2/neu status of tumour tissue.

Informed consent was obtained from all the participants prior to the commencement of the study. Data was retrieved from histopathology reports of the patients. Identification details of the participants were not revealed, and a coding system was used at every step to ensure confidentiality. Reporting of immunohistochemical results were according to international recommendations [7, 8] and the laboratory procedures were of the highest standard as per the accreditation guidelines of the College of American Pathologists.

All statistical analyses were performed using Graphpad Prism version 8.0 (GraphPad Software Inc, San Diego, CA, USA). Quantile-quantile plots and the Shapiro-Wilk test were used to assess the normality of data (13) and non-parametric tests (Mann-Whitney test) were used to analyse datasets which are not normally distributed. Continuous data are

summarised as mean and standard deviation. Statistical significance is defined as $p < 0.05$.

3. Results

A total of 92 female patients were included in this study. The mean age of the patients with breast carcinoma was 53.99 years. The cohort of breast carcinoma comprised of different histological types.

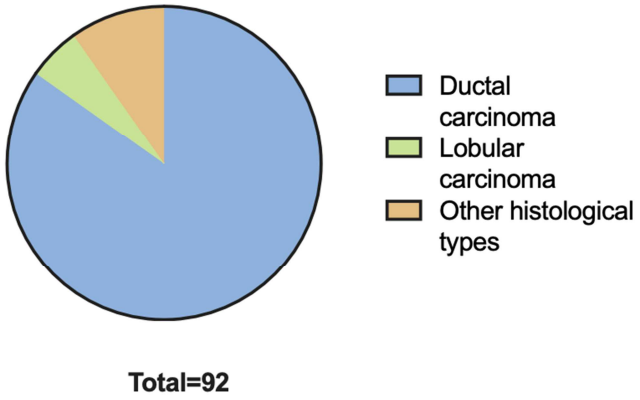

Figure 1. *Histological types of breast carcinoma.*

Invasive ductal carcinoma was the most common histological type. There were 78 (84.78%) cases out of 92, classified as invasive ductal carcinoma. Invasive lobular type of breast carcinoma included 5 cases (5.44%) and the remaining 9 (9.78%) were less common histological types such as mucinous carcinoma, invasive papillary carcinoma, invasive cancer with medullary carcinoma-like features, and cribriform carcinoma (figure 1).

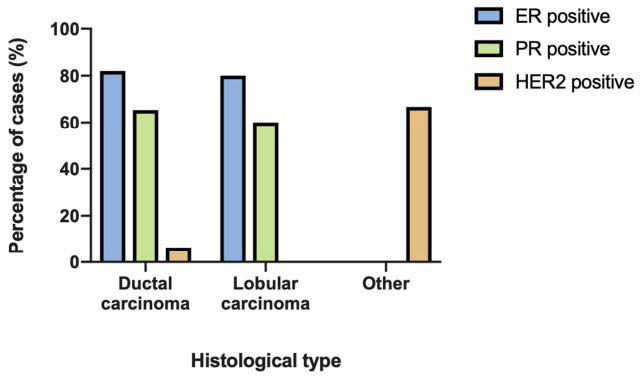

Figure 2. *ER, PR and HER 2/neu status of breast carcinoma.*

Figure 2 in the invasive ductal carcinoma group 64 (82.05%) were ER-positive, 51 (65.39%) were PR-positive and 5 (6.41%) were HER2/neu-positive. Of the patients with invasive lobular carcinoma 4 (80.0%) were ER-positive, 3 (60.0%) were PR-positive and none were HER2/neu positive. Of the patients with "other histological types", none had ER-positive or PR-positive disease and 66.67% (n = 6) were HER2/neu-positive. Triple negative disease was seen in 33.33% of patients in the group with "other histological types".

Figure 3 in the cohort of breast carcinoma 29 patients (31.52%) had node positive disease while 63 patients (68.48%) had node negative disease. 6 (20.69%) of the node positive cases were HER 2/neu -positive compared to 5 (7.94%) of the node negative cases which were HER 2/neu-positive. This difference was not statistically significant ($p = 0.629$) when the two groups were compared using a Mann-Whitney test.

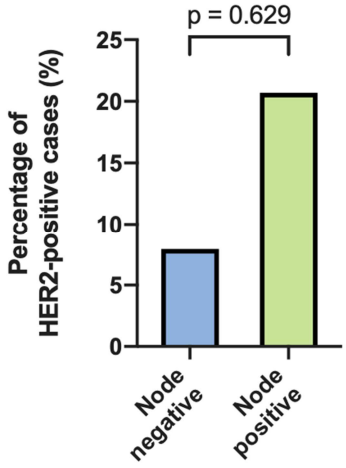

Figure 3. *Association between HER 2/neu status and lymph nodal involvement.*

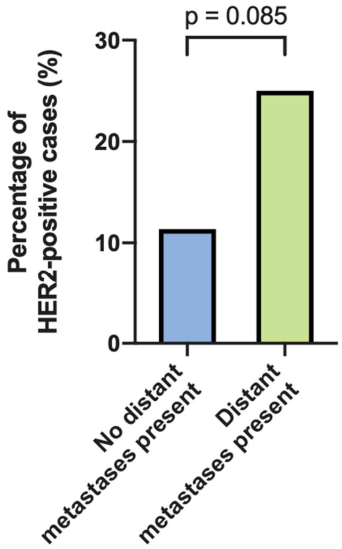

Figure 4. *Association between HER 2/neu status and distant metastases.*

Figure 4 in the cohort of breast carcinoma 4 patients (4.35%) had distant metastases whilst 88 patients (95.65%) had no distant metastases. 1 (25.00%) of the patients with distant metastases had HER2-positive disease compared to 10 (11.36%) of the patients with no distant metastases who were HER2-positive. This difference was not statistically significant ($p = 0.085$) when the two groups were compared using a Mann-Whitney test.

Correlation analyses of ER, PR, HER 2/neu status and Ki 67 proliferative index

As the data were found to be non-parametric when

assessed using Q-Q plots and the Shapiro-Wilk test, the Spearman's rank correlation test was used to perform all correlation analyses. Spearman's rho and associated p values are reported in each case.

ER status showed a significant negative correlation with HER2/neu status (rho = -0.634, $p < 0.0001$). PR status showed a significant negative correlation with HER2/neu status (rho = -0.834, $p < 0.0001$). Ki 67 index showed a significant positive correlation with HER2/neu status (rho = 0.248, p = 0.017).

4. Discussion

Study included 92 cases of invasive breast carcinoma. Mean age was 53.99 years (standard deviation 8.03) and the age range was 38 to 76 years. Studies done in different parts of the world show great variation in the mean age of breast carcinoma. It has been reported by Adesunkanmi ARK et al, that in the African region the mean age was 48 years and the majority were premenopausal [14]. In the European continent the results were somewhat different [15] as majority of women were postmenopausal at the initial presentation. Bowen RL et al [16], in their study done in the UK has shown the median age at presentation for black women is same as for African women. The age at presentation for African-American women is significantly younger than their Caucasian counterparts [17]. There may be several reasons for the above findings including the varied expression of breast cancer associated molecular genetics, most importantly BRCA 1 and 2 genes [18].

In the cohort of breast cancer invasive ductal carcinoma was the most common histological type (84.78%). There were five cases (5.44%) out of 92 classified as invasive lobular carcinoma and the remaining 9.78% included the less common variants such as mucinous carcinoma, cribriform carcinoma, papillary carcinoma and carcinoma with medullary features [19, 20]. There was diversity in the expression of ER, and only 73.91% were reported as ER positive by immunohistochemical staining [21]. PR positivity was seen in 58.69% cases and 11.95% were reported as HER2/neu, 3+/positive. Of the invasive ductal carcinomas, 82.05% were ER positive while 80% of invasive lobular carcinoma showed positive results for ER [22]. Potemski et al. (2007) has shown that the higher the level of receptor expression [23], the lesser the adverse outcomes. PR expression is taken as a factor increasing the predictive value of ER. There was no statistically significant association between ER positivity and the histological type of breast carcinoma ($p< 0.05$).

HER2/neu positivity was seen in 6.41% of ductal carcinoma. None of the invasive lobular cancers were HER2/neu positive. In the remaining patients 66.67% were HER2/neu positive. HER2/neu expression which is known to have prognostic and predictive implications vary between 15-30% in invasive carcinoma [10]. In the current study HER2/neu positivity was found only in 11.95% cases which is lower than the expected result. Breast cancer cells can have up to 100- fold increase in HER2/neu protein resulting in marked overexpression of receptors on tumour cells [24].

The study had 29 patients (31.52%) with node positive disease at the time of presentation. 63 (68.48%) patients were free of nodal involvement at the time of the diagnosis of breast carcinoma. 6 of the node positive cases (20.69%) were HER2/neu positive. There was no statistically significant difference between the HER2/neu positivity in node positive and node negative groups (p=0.629). Distant metastasis was seen in only 4 out of 92 patients (4.35%). HER 2/neu was positive in 25% of patients with distant metastasis and 11.36% of patients with no distant metastasis. This difference was not statistically significant (p=0.085). Gabos Z. et al, [25] in their study have observed the serious prognostic implications of human epidermal growth factor receptor including the risk of brain metastasis. Horiguchi J, Koibuchi Y, Iijima K, et al, [26] have discussed about the co-expression of ER and HER2 as a predictive factor for response to treatment.

5. Conclusion

In the cohort of breast cancer, the most common histological type was invasive ductal carcinoma. Mean age at presentation was 53.99 years although there is considerable variation in different parts of the world. More extensive collaborative research would be useful to identify the risk factors of breast cancer in different ethnic groups. Evaluation of ER, PR and HER2/neu status and Ki 67 proliferative index are performed routinely in breast carcinoma, in view of the significant clinical implications [27]. ER (+) tumours have more favourable outcome in comparison to ER (-) tumours [28]. HER2/neu positive status is associated with an aggressive behaviour and shorter disease free interval [29].

According to the results of the current study ER status showed a significant negative correlation with HER2/neu status (rho = -0.634, $p < 0.0001$). PR status showed a significant negative correlation with HER2/neu status (rho = -0.834, $p < 0.0001$). Ki 67 index showed a significant positive correlation with HER2/neu status (rho = 0.248, p = 0.017).

Conflicts of Interest

Authors declare that there are no conflicts of interests.

Data Availability & funding: Data was collected from the laboratory data base, accessible to the permanent staff of the department. Research project was self-funded.

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References

[1] Ferlay J, Colombet M, Soerjomataram I, Mathers C, Parkin DM, Pineros M, Znaor A, Bray F. Estimating the global cancer incidence and mortality in 2018: GLOBOCAN sources and methods. Int. J. Cancer. 2019; 144: 1941-1953.

[2] Ferlay J, Soerjomataram I, Dikshit R, Eser S, Mathers C, Rebelo M, et al. Cancer incidence mortality worldwide: Sources, methods and major patterns in GLOBOCAN 2012. Int J Cancer. 2015; 136: E359–86.

[3] Steven S. Coughlin, Donatus U. Ekwueme. Breast cancer as a global health concern. Cancer Epidemiology. 2009; 33 (5): 315-318. doi.org/10.1016/j.canep.2019.10.003.

[4] Pesce K, Orruma MB, Hadad C, Cano YB, Secco R, Cernadas A. BI-RADS Terminology for Mammography Reports: What Residents Need to Know. RadioGraphy. 2019; 39 (2): online doi.org/10.1148/rg.2019180068 From the Department of Breast Radiology, Hospital Italiano de Buenos Aires, Pres. Tte. Gral. Juan Domingo Perón 4190, Capital Federal C1199 ABB, Argentina. Address correspondence to K. P.

[5] Pustahija AH, Ivanac G, Brkljacic B. US and MRI in the evaluation of mammographic BI-RADS 4 and 5 microcalcifications. Diagn Intery Radiol. 2018; 24 (4): 187-194. doi: 10.5152/dir.2018.17414.

[6] Badowska-Kozakiewicz AM, Patera J, Sobol M, Przybylski J. The role of oestrogen and progesterone receptors in breast cancer – immunohistochemical evaluation of oestrogen and progesterone receptor expression in invasive breast cancer in women. Contemp. Oncol (Pozn). 2015; 19 (3): 220-225. doi: 10.5114/wo.2015.51826.

[7] Iqbal BM, Buch A. Hormone receptor (ER, PR, HER2/neu) status and proliferation index marker (Ki-67) in breast cancers: Their onco-pathological correlation, shortcomings and future trends. Med J DY Patil Univ. 2016; 9: 674-9. doi: 10.4103/0975-2870.194180.

[8] Moasser MM. The oncogene HER2; its signaling and transforming functions and its role in human cancer pathogenesis. Oncogene. 2007; 26 (45): 6469-6487.

[9] Abubakar M, Figueroa J, Ali HR, Blos F, Lissowska J, Caldas C, Easton DF, Sherman ME, Garcia-Closas M, Dowsett M, Pharoah PD. Combined quantitative measures of ER, PR, HER2, and KI67 provide more prognostic information than categorical combinations in luminal breast cancer. Modern Pathology.2019; 32: 1244-1256.

[10] Burstein HJ. The distinctive nature of HER2-positive breast cancers. N Eng J Med. 2005; 353 (16): 1652-1654.

[11] Mohammed RA, Ellis IO, Lee AH, Martin SG. Vascular invasion; an overview of recent prognostic developments & molecular pathophysiological mechanisms. Histopathology. 2009; 55: 1-9.

[12] Arteaga CL, Sliwkowski MX, Osborne CK, perez EA, Puglisi F, Gianni Luca. Treatment of HER2-positive breast cancer: current status and future perspectives. Nature Reviews Clinical Oncology. 2012; 9: 16–32.

[13] Henderson AR. Testing experimental data for univariate normality. Clin Chim Acta. 2006; 366 (1–2): 112–29.

[14] Adesunkanmi ARK, Lawal OO, Adelusola KA, Durosimi MA. The severity, outcome and challenges of breast cancer in Nigeria. Breast. 2006; 15 (3): 399–409. [PubMed] [Google Scholar].

[15] Health Wales NHS Trust Public. WCISU Annual Publication No. SA10/01: Cancer Incidence in Wales 2004–2008. Welsh Cancer Intelligence and Surveillance Unit, Cardiff, UK, 2010, http://www.wales.nhs.uk/sites3/Documents/242/incpub2010.pdf.

[16] Bowen RL, Duffy SW, Ryan DA, Hart IR, Jones JL. Early onset of breast cancer in a group of British black women. British Journal of Cancer. 2008; 98 (2): 277–281. [PMC free article] [PubMed] [Google Scholar].

[17] Elmore JG, Moceri VM, Carter D, Larson EB. Breast carcinoma tumor characteristics in black and white women. Cancer. 1998; 83 (12): 2509–2515. [PubMed] [Google Scholar].

[18] Haffty BG, Silber A, Matloff E, Chung J, Lannin D. Racial differences in the incidence of BRCA1 and BRCA2 mutations in a cohort of early onset breast cancer patients: African American compared to white women. Journal of Medical Genetics. 2006; 43 (2): 133–137. [PMC free article] [PubMed] [Google Scholar].

[19] WHO Classification of tumours of the Breast, 4th Edition, ISBN -13: 978-9283224334.

[20] Rambau PF, Chalya PL, Manyama MM, Jackson KJ. Pathological features of Breast Cancer seen in Northwestern Tanzania: A nine years retrospective study. BMC Research Notes. 2011: p. 214. [PMC free article] [PubMed] [Google Scholar].

[21] Yager JD, Davidson NE. Estrogen Carcinogenesis in Breast Cancer. N Eng J Med. 2006; 354: 270-282. doi: 10.1056/NEJMra050776.

[22] Francis GD, Dimech M, Giles L et al. Frequency and reliability of oestrogen receptor, progesterone receptor and HER2 in breast carcinoma determined by immunohistochemistry in Australasia: Results of the RCPA Quality Assurance Program. J Clin Pathol 60: 1277–1283. 2007. [Crossref] [Google Scholar].

[23] Potemski P, Kusińska R, Kubiak R, et al. The predictive value and prognostic significance of the degree of expression of steroid receptors in breast cancer operation. Oncol Pol. 2007; 10: 49–54. [Google Scholar].

[24] Park K, Han S, Kim HJ, Kim J, Shin E. HER2 status in pure ductal carcinoma in situ and in the intraductal and invasive components of invasive ductal carcinoma determined by fluorescence in situ hybridization and immunohistochemistry. Histopathology. 2006; 48 (6): 702-707. [Google Scholar].

[25] Gabos Z, Sinha R, Hanson J et al. Prognostic significance of human epidermal growth factor receptor positivity for the development of brain metastasis after newly diagnosed breast cancer. Journal of clinical oncology. 2006; 24 (36): 5658-5663. [Google scholar].

[26] Horiguchi J, Koibuchi Y, Iijima K, et al. Co-expressed type of ER and HER2 protein as a predictive factor in determining resistance to antiestrogen therapy in patients with ER-positive and HER2-positive breast cancer. Oncol Rep. 2005; 14: 1109–1116. [Google Scholar].

[27] Allemani C, Sant M, Berrino F, et al. Prognostic value of morphology and hormone receptor status in breast cancer – a population-based study. Br J Cancer. 2004; 91: 1263–8. [PubMed] [Google Scholar].

[28] Tsutsui S, Ohno S, Murakami S, Hachitanda Y, Oda S. Prognostic value of epidermal growth factor receptor (EGFR) and its relationship to the estrogen receptor status in 1029 patients with breast cancer. Breast Cancer Res Treat. 2002; 71: 67–75. [PubMed] [Google Scholar].

[29] Barzanti F, Dal Susino M, Volpi A, et al. Comparison between different cell kinetic variables in human breast cancer. Cell Prolif. 2000; 33: 75–89. [PubMed] [Google Scholar].