

Correlation of Hormone Receptor and Human Epidermal Growth Factor Receptor-2/neu Expression in Breast Cancer with Various Clinicopathologic Factors

Abstract

Background: A significant development in the breast carcinoma management is the correlation between the presence of hormone receptors in the tumor and response to hormonal therapy and chemotherapy. Human epidermal growth factor receptor-2/neu (Her-2/neu) overexpression also serves as a very useful parameter to predict response to herceptin. **Aim of Study:** The study was conducted to correlate immunohistochemical expression of markers such as estrogen receptor (ER), progesterone receptor (PR), and Her-2/neu with various clinicopathologic parameters. **Materials and Methods:** The study included 509 cases of breast carcinoma over a period of 5 years (from May 2009 to May 2014). Immunohistochemistry (IHC) for ER, PR, and her-2/neu was performed. **Results:** ER positivity was observed in 42.8% (218/509) cases, PR positivity in 31.8% (194/509) cases whereas her-2 neu positivity was seen in 40.7% (203/509) cases. Triple marker (ER, PR, and Her-2/neu) negative cases were 23.6% (120/509) cases. ER and PR expression was found to have a statistically significant correlation with tumor grade. Statistically significant correlation was observed between tumor size and tumor grade and her-2/neu expression. Her-2/neu expression showed statistically significant association with tumor stage. As the tumor grade increased, the proportion of triple-negative cases went on increasing, which was statistically significant. **Conclusion:** IHC has an increasingly important prognostic role in determination of factors that affect clinicopathologic features. Nevertheless, the results of this large series showed different patterns of findings with respect to clinicopathologic features.

Keywords: Breast cancer, estrogen receptor, human epidermal growth factor receptor-2/neu, hormone receptors, progesterone receptor

Introduction

Breast cancer is the most common malignancy in females worldwide, and more than 1 million women are diagnosed with breast cancer each year.^[1] Most cases of invasive carcinoma breast are ductal in origin (over 90%). Invasive ductal carcinoma of no special type not otherwise specified (NOS) accounts for 60%–80% of all cases of breast carcinoma.^[2]

In breast carcinoma, several features have prognostic significance including histologic subtype, grade, lymph node states, estrogen receptor (ER)/progesterone receptor (PR) status, human epidermal growth factor receptor-2/neu (her-2/neu) status, growth factors and its receptors, proliferative activity and DNA content, oncogenes, and tumor suppressor genes. At present, ER status is regarded as the most powerful

predictive marker in the treatment of breast cancer even though ER and PR are codependent variables.^[2]

In today's era, a conservative cum reconstructive surgical approach is becoming more and more popular in the treatment of breast carcinoma. A correlation between the presence of hormone receptors in the tumor and response to hormonal therapy and chemotherapy is a significant development in the breast carcinoma management. Her-2 neu overexpression also serves as a very useful parameter to predict response to herceptin, but it is not a good predictor of response to chemotherapy or overall survival.^[2,3] This study was conducted to correlate immunohistochemical expression of markers such as ER, PR, and Her-2neu with various clinicopathologic parameters.

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Materials and Methods

The present study included all cases of breast carcinoma (509) over a period of 5 years (from May 2009 to May 2014) retrieved from the archives of Department of Pathology of our institute. The study was approved by the Institutional Ethics Committee. Clinical characteristics of patients such as age, sex, and menopausal status were documented from case files. All the modified radical mastectomy specimens were examined grossly to look for tumor size and nodal metastasis. All tissues were fixed in 10% buffered formalin immediately after resection but not more than 24 h. Representative sections were taken from tumor and submitted for processing, and routine hematoxylin and eosin staining was performed for histopathological diagnosis. Tumors were graded according to Modified Bloom Richardson grading system.

Immunohistochemistry (IHC) for ER, PR, and her-2/neu was performed on representative blocks of paraffin-embedded tumor tissue. Four micrometers thick sections were taken on poly-L-lysine-coated slides and submitted for IHC. Antigen retrieval was done using citrate buffer at pH 2.5 for hormone receptors and pH 6 for her-2/neu. They were then incubated for 30 min with primary monoclonal antibodies against her-2 (DAKO, clone 124, 1:100), ER (DAKO, clone 1D5, 1/25), and PR (DAKO, clone PgR636, 1/50), followed by incubation with biotin-labeled secondary antibodies. The streptavidin-peroxidase complex was visualized using diaminobenzidine as a chromogenic substrate. The normal breast ducts served as internal positive control for ER/PR. Breast carcinoma with known her-2 neu overexpression served as an external positive control for her-2/neu staining.

ER or PR was considered positive when more than 1% of tumor cell nuclei were immunoreactive. ER or PR were considered negative if <1% of tumor cell nuclei were immunoreactive in the presence of evidence that the sample can express ER or PR (positive intrinsic controls seen). Uninterpretable for ER or PR if finding that no tumor nuclei are immunoreactive and that internal epithelial elements present in the sample or separately submitted from the same sample lack any nuclear staining.

For interpretation of her-2/neu staining, the following method was used:^[2]

- Score 0 (Negative): No staining is observed or membrane staining is observed in <10% of the tumor cells
- Score 1+ (Negative): A faint/barely perceptible membrane staining is detected in more than 10% of the tumor cells. The cells are only stained in part of their membrane
- Score 2+ (weakly Positive): A weak-to-moderate complete membrane staining is observed in more than 10% of the tumor cells
- Score 3+ (Strongly Positive): A strong complete membrane staining is observed in more than 30% (formerly 10%) of the tumor cells

- Score 3 + was considered as positive immunostaining for her-2 neu.

Results

The study group comprised of a total of 509 breast carcinoma patients, majority being females (501/509; 98.4%). Out of these, 90 were below 40 years of age whereas 419 were more than 40 years of age. According to tumor size, most of the cases (56.2%) belonged to intermediate group with tumor size of 2–5 cm. The most commonly encountered histologic type was infiltrating duct carcinoma, not otherwise categorized (invasive ductal carcinoma [IDC], NOC). Most of the tumors (64%) belonged to modified Richardson–Bloom (MRB) Grade 2. Intratumoral and peritumoral lymphocytes were observed in 26.1% cases whereas lymph node metastasis was detected in 54.2% cases (276/509). Skin infiltration by tumor was present in 39 cases whereas distant metastases were detected in 13 cases (2.6%). The detailed clinicopathological profile of the study group is depicted in Table 1.

Among the carcinoma breast patients, majority belonged to Stage II (248/509) followed by Stage III (150/509), Stage I (98/509), and Stage IV (13/509).

Immunohistochemical profile of the study group as shown in Table 2 revealed ER positivity in 42.8% (218/509) cases, PR positivity in 31.8% (194/509) cases whereas her-2 neu positivity seen in 40.7% cases [Figure 1]. Triple marker (ER, PR, and Her-2/neu) negative cases were 23.6% and 8.8% were triple marker positive. Cases in which both ER and PR showed similar results (that is, either both positive or both negative) were considered to be concordant whereas cases with one marker positive and other negative or vice versa were taken as discordant. In the present study, 85.9% cases were concordant, of which 33.4% showed both ER and PR positivity whereas 52.5% showed both markers as negative. Nearly 14.1% cases were found to show discordant results.

The correlation between various clinicopathological parameters and immunohistochemical profile is shown

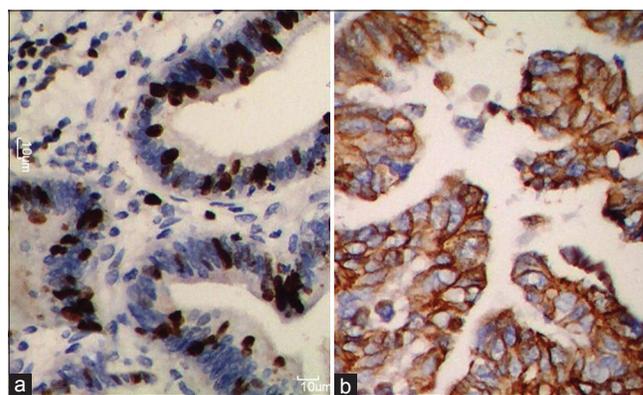


Figure 1: (a) Photomicrograph shows strong estrogen receptor positivity in the tumor nuclei; (b) strong membranous staining for human epidermal growth factor receptor-2 (score 3+)

Table 1: Clinicopathological profile of breast carcinoma patients

	<i>n</i> (%)
Gender	
Female	501 (98.4)
Male	8 (1.6)
Age group	
<40	90 (17.7)
>40	419 (82.3)
Side	
Left	243 (47.7)
Right	255 (50.1)
Bilateral	10 (2.0)
Menopausal status	
Premenopausal	246 (44.8)
Postmenopausal	255 (55.2)
Religion	
Hindu	460 (90.4)
Muslim	42 (8.3)
Sikh	52 (1)
Christian	2 (1.4)
Tumor size	
≤2	175 (34.4)
2-5	286 (56.2)
>5	48 (9.4)
Tumor type	
IDC	487 (95.5)
ILC	8 (1.6)
Colloid	4 (0.4)
Medullary	2 (0.4)
Papillary	2 (0.4)
Metaplastic	1 (0.2)
<i>In situ</i> component	
Absent	313 (61.5)
Present	162 (31.8)
Not mentioned	34 (6.7)
MRB grade	
I	20 (3.9)
II	326 (64.0)
III	146 (28.7)
Not mentioned	17 (3.3)
Lymph node	
Absent	233 (45.8)
Present	276 (54.2)
Skin infiltration	
Absent	470 (92.4)
Present	39 (7.7)
Metastasis	
Absent	496 (97.4)
Present	13 (2.6)
Intratumoral and peritumoral lymphocytes	
Absent	346 (68)
Present	133 (26.1)
Not mentioned	30 (5.9)

IDC – Invasive ductal carcinoma; ILC – Invasive lobular carcinoma; MRB – Modified Richardson Bloom

Table 2: Immunohistochemical profile of the study group

	Status	<i>n</i> (%)
ER	Negative	291 (57.2)
	Positive	218 (42.8)
PR	Negative	315 (61.9)
	Positive	194 (31.8)
HER2	Negative	287 (57.5)
	Positive	203 (40.7)
	Equivocal	9 (1.8)
Triple	Negative	120 (23.6)
	Positive	45 (8.8)
Concordant	437 (85.9)	ER+/PR+ 170 (33.4)
		ER-/PR- 267 (52.5)
Discordant	72 (14.1)	ER+/PR- 48 (66.7)
		ER-/PR+ 24 (33.3)

ER – Estrogen receptor; PR – Progesterone receptor; HER2 – Human epidermal growth factor receptor 2

in Table 3. On correlating age with ER positivity, the difference between women <40 years and those >40 years was found to be statistically significant ($P = 0.04$). Moreover, as the MRB grade of the tumor increased, the ER positivity gradually decreased ($P < 0.001$). However, no correlation was observed between ER expression and menopausal status, tumor size, or nodal status. Similar to ER expression, the PR expression was also found to have a statistically significant correlation with MRB grade of tumor ($P < 0.001$). Rest of the parameters did not have any significant association with PR positivity.

Her-2/neu expression of tumor decreased with increase in the tumor size which was statistically significant ($P = 0.009$). Moreover, statistically significant correlation was observed between tumor grade and her-2/neu expression. There was no significant association of her-2/neu positivity with age, menopausal status, or node involvement by tumor.

On correlating the triple negative and triple positive cases with the clinicopathological parameters, it was observed that as the MRB grade of the tumor increased, the proportion of triple negative cases went on increasing (60% in Grade 1, 66.3% in Grade 2, and 86.4% in Grade 3), which was statistically significant ($P = 0.01$). However, none of the other parameters showed any significant association with triple marker positivity or negativity.

The correlation of various parameters with the concordant and discordant cases is shown in Table 4. The difference between ER/PR expression (among concordant cases) between women <40 years and >40 years was found to be statistically significant ($P = 0.04$). The ER/PR expression in the concordant cases also correlated significantly with MRB grade of tumor ($P < 0.01$). However, among the discordant cases, no significant association was observed with any of the clinicopathological parameters.

When the tumor stage was correlated with various immunohistochemical markers, her-2/neu expression

Table 3: Correlation between various clinicopathological parameters and immunohistochemical profile

	ER			PR			HER2			
	ER+	ER-	Significant (P)	PR+	PR-	Significant	HER2+	HER2-	Equivocal	Significant
Age										
<40	30	60	0.04	28	62	0.15	37	49	3	0.44
>40	188	231		166	253		166	238	6	
Menopausal status										
Premenopausal	96	150	0.14	89	157	0.58	97	141	6	0.54
Postmenopausal	116	139		99	156		104	140	3	
Tumor size										
≤2	74	101	0.68	69	106	0.74	76	93	4	0.009
2-5	126	160		109	177		116	160	2	
>5	18	30		16	32		11	34	3	
MRB grade										
I	14	6	<0.001	10	10	<0.001	5	12	2	0.01
II	153	173		139	187		138	176	4	
III	42	104		36	110		55	88	2	
Node										
Absent	95	138	0.41	89	144	1.0	100	124	6	0.19
Present	123	153		105	171		103	163	3	

ER – Estrogen receptor; PR – Progesterone receptor; HER 2 – Human epidermal growth factor receptor 2; MRB – Modified Richardson Bloom

Table 4: Correlation of clinicopathological parameters with the concordant and discordant cases

	Total cases			Concordant cases			Discordant cases		
	Total cordant cases	Total discordant cases	Significant	ER+/PR+	ER-/PR-	Significant	ER+/PR-	ER-/PR+	Significant
Age									
<40	74	16	0.3	21	53	0.04	9	7	0.37
>40	363	56		149	214		39	17	
Menopause status									
Premenopausal	209	37	0.52	74	135	0.23	22	15	0.2
Postmenopausal	222	33		91	131		25	8	
Tumor size (cm)									
≤2	156	19	0.08	62	94	0.78	12	7	0.88
2-5	237	49		93	144		33	16	
>5	44	4		15	29		3	1	
MRB grade									
I	16	4	0.59	10	6	<0.01	4	0	0.35
II	278	48		122	156		31	17	
III	128	18		30	98		12	6	
Node									
Absent	203	30	0.52	77	126	0.76	18	12	0.3
Present	234	42		93	141		30	12	

ER – Estrogen receptor; PR – Progesterone receptor; MRB – Modified Richardson Bloom

decreased with increase in stage and the difference was statistically significant ($P = 0.04$). There was no significant association between ER and PR expression or triple negative cases and tumor stage.

Discussion

A correlation between the presence of hormone receptors in the tumor and response to hormonal therapy and chemotherapy is a significant development in the breast carcinoma management. At present, ER status is regarded

as the most powerful predictive marker in the treatment of breast cancer even though ER and PR are codependent variables.^[2,3]

Hormone receptors were initially measured by radioligand binding assay on tissue cytosol, but this has been effectively taken over by IHC. They can also be assessed by *in situ* hybridization and polymerase chain reaction. IHC has the advantages that it does not require fresh tissue, can be performed even on minute quantities of tissues, and is a relatively easy technique. ER is a thermolabile unstable

protein, water soluble with a short half-life after surgical resection, so it is very important to ensure rapid fixation of specimen to obtain appropriate results. Several authors have attempted to standardize the technique and method of reporting to bring some semi-quantitation to the reporting of IHC. Scoring systems have been devised to express the results incorporating two features: number of tumor cell nuclei which are stained and the intensity of staining.^[2,3]

Immunohistochemical profile showed ER positivity in 42.8% cases, PR positivity in 31.8%, and Her-2neu positivity in 40.7% cases. Immunohistochemically, 23.6% of all 509 cases were triple negative. These findings are analogous to other similar studies done by Ayadi *et al.*,^[4] Ahmed *et al.*,^[5] and Vasudha *et al.*^[6] On the contrary, studies done by Lal *et al.*,^[7] Moser Emliroise *et al.*,^[8] Vaidhyanatha *et al.*,^[9] and Munjal *et al.*^[10] depicted a high percentage of Her-2/neu reactivity.

Hormone receptor expression has not been found to correlate well with histological type of breast carcinoma (ductal vs. lobular, no significant association). However, breast cancers with negative ER generally have pushing margins, Grade 3 histology, comedo type necrosis, lymphoid stroma, and central necrosis/fibrosis.^[11] ER concentrations are usually lower in tumors in premenopausal women compared to postmenopausal.^[12]

In the subset of patients with ER positivity, the difference between women <40 years and those >40 years was found to be statistically significant ($P = 0.04$). ER positivity was found to have significant relation with tumor grade. A number of studies conducted by Ayadi *et al.*,^[4] Adebamowo *et al.*,^[13] Lu *et al.*,^[14] Pinto *et al.*,^[15] Looi and Cheah,^[16] and Kaptain *et al.*^[17] support our findings. Relationship with menopausal status was not found to have a significant association with ER positivity in coherence with findings of a study conducted by Ahmed *et al.*^[5]

No significant relation was noted between ER positivity and tumor size, similar to the studies conducted by Ahmed *et al.*,^[5] Bamberger *et al.*,^[18] and Kiliç and Yaldiz.^[19] Similarly, no significant relation was seen between ER positivity and lymph node positivity, a finding which is supported by many studies, i.e., by Prati *et al.*,^[20] Huang *et al.*,^[21] Vasudha *et al.*,^[6] and Azizun-Nisa *et al.*^[22]

Regarding PR positivity, it had a significant association with only tumor grade while with all other clinicopathologic parameters such as age, menopausal status, tumor size, and lymph node status, no significant relation was noted.

Her-2/neu (c-erb B2) is an oncogene that encodes a transmembrane glycoprotein with tyrosine kinase activity and belongs to the epidermal growth factor receptor family. Her-2 neu overexpression has been observed in many cases of carcinoma breast; moreover, with the discovery of herceptin (trastuzumab) as a therapeutic agent, the assessment of her-2 neu amplification in all

breast cancer patients has become almost mandatory. Her-2 neu overexpression can be measured by either IHC or fluorescent *in situ* hybridization (FISH). There is an ongoing controversy regarding the usefulness of the two techniques; however, a consensus has now been reached that the best, cost-effective approach is to begin with IHC and do grading. If the results are either 0 or 3+, there is no need to perform FISH as the results correlate with gene expression. However, 1+ or 2+ results need to be confirmed using FISH.^[2,3]

Her-2 neu overexpression serves as a very useful predictor of response to herceptin, but it is not a good predictor of response to chemotherapy or overall survival.

Regarding the relationship of her-2 neu with the histological types of breast cancer, its overexpression is seen in almost all cases of high-grade ductal carcinoma *in situ*, in 20%–30% IDC and small percentage of invasive lobular carcinoma.^[23] On the contrary, it is characteristically absent in tubular and Grade 1 carcinomas.^[24]

There is an inverse correlation between her-2/neu amplification and hormone receptor (ER and PR) expression. As in our study, ER and PR reactivity inverse association with Her-2neu is supported by studies conducted by Ahmed *et al.*,^[5] Ayadi *et al.*,^[4] Almasii *et al.*,^[25] Ranatunga *et al.*,^[26] Vasudha *et al.*,^[6] Huang *et al.*,^[21] and Rashed *et al.*^[27]

With regard to Her-2neu positivity, a significant relation to tumor size was noted similar to the findings of studies conducted by Almasii *et al.*^[25] and Vasudha *et al.*^[6] On the contrary, majority of the studies conducted by Ayadi *et al.*,^[4] Prati *et al.*,^[20] Aliga *et al.*,^[28] and Huang *et al.*^[21] do not support this finding.

Her-2neu positivity in our study showed a significant association with tumor grade; a finding supported by Rashed *et al.*,^[27] Cho *et al.*,^[29] and Moradi-Marjaneh *et al.*^[30] However, Al-Moundhii *et al.*^[31] and Yamashita *et al.*^[32] had contradictory findings.

In our study, Her-2neu reactivity did not have a significant association with age and menopausal status, which was in accordance with Al Moundhii *et al.*^[31] and Yamashita *et al.*^[32] No significant correlation was observed between her-2 neu overexpression and lymph node status as documented by Vasudha *et al.*,^[6] Huang *et al.*,^[21] and Azizun-Nisa *et al.*,^[22] whereas Hussein *et al.*^[33] had contrary findings.

There is a considerable overlap between triple-negative tumors and basal-like cancers, but still these are not synonymous with each other. Only 77% of cases classified by gene expression profiling as basal like show a triple negative phenotype whereas only 72% of cases of triple negative cancers exhibit a basal-like gene expression profile. Triple-negative tumors represent a distinct

category of tumors on account of usually high-grade Intraductal Carcinoma Not Otherwise Specified (IDC NOS) morphology, a high degree of aneuploidy, a greater tendency for lung and brain metastasis, and thereby exhibit a poorer prognosis. In the present study, the triple negative tumors had significant relation with tumor grade.^[34]

Limitations of the present study are that IHC is not an ideal technique to assess her 2 neu, especially in the cases with weak positivity which should be confirmed by better methods such as FISH. However, in developing countries like India, IHC being universally available (at least in major centers) is still most widely used.

IHC has an increasingly important prognostic role in determination of factors that affect clinicopathologic features. This is a large series on immunohistochemical profile of carcinoma breast as well as their correlation with clinicopathological parameters in Indian population. In the present study, ER and PR expression was found to have a statistically significant correlation with tumor grade. Statistically significant correlation was observed between tumor size and tumor grade and her-2/neu expression. Her-2/neu expression showed statistically significant association with tumor stage.

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Conflicts of interest

There are no conflicts of interest.

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