Original Research Article

Expression of ER, PR and HER 2 Receptor Status in Breast Carcinomas and its Correlation with Histopathological Grading

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Abstract

Introduction: Breast cancer is the most common cancer in women in developed countries. In India; it is second to cancer of cervix among women. Several histopathological features have prognostic significance in breast carcinoma which includes histologic subtype, grade, lymph node status, ER/PR status, Growth factors and its receptors, proliferation activity and DNA content, oncogenes and tumor suppressor genes. The Immunohistochemistry (IHC) classification provides both therapeutic and prognostic information.

Aim and objectives: To assesses the ER/PR status and HER -2 status of breast carcinoma, to correlate the ER/PR and HER-2 receptors status with modified Bloom- Richardson histological grading, and clinico pathological parameters.

Materials and methods: This study was undertaken over a period of 2 years from August 2013 to July 2015 in the Department of Pathology, Chalmeda Anand Rao Institute of Medical Sciences. Total of 75 cases of carcinoma breast in females diagnosed histopathologically, belonging to age group of 21-85 years were included in the study.

Results: Histopathological grading was done according to Modified Bloom Richardson's grading. 27(36%) cases were of grade II and 24(32%) cases each were of grade I and grade III. ER and PR were positive in 76% and 58.7% of tumors respectively. HER-2 over expression (score 2+ & 3+) was seen in 44% of tumors and was negative (0 or 1+) in 56% of tumors. Among the most common histologic subtype i.e., IDC (NOS), ER, PR and HER-2 were expressed in 75.36%, 60.86% and 44.92% respectively. Tumors with ER/PR-, HER2+ subtype were larger (>5cms), 1(1.3%) each medullary and apocrine carcinoma were positive for HER-2. None of the lobular, mucinous and papillary carcinoma expressed HER-2. Statistically significant values were noted for histologic grade immunohistochemical sub types, lympho vascular invasion and nuclear pleomorphism.

Conclusion: In this study ER, PR and HER-2 receptors status correlates well with histopathological grading and other clinico-pathological parameters. These assays have the advantage of allowing only tumor cells to be assessed for receptor status. They can be conducted relatively inexpensively on routinely processed tissue sections with no need for specialized equipment.

Key words

Carcinoma Breast, ER, PR, HER2 receptors.

Introduction

Breast cancer is the most common cancer in women in developed countries [1], In India; it is second to cancer cervix among women. It is estimated that approximately 80,000 cases occur annually; the age adjusted incidence rates varying between 16 and 25/ 100,000 population [2]. The increase in incidence rate is attributed to newer diagnostic modalities like mammography, sonography, needle aspiration cytology, core biopsy and mainly because of increasing awareness in the country.

Several histopathological features have prognostic significance in breast carcinoma which includes histologic subtype, grade, lymph node status, ER/PR status, Growth factors and its receptors, proliferation activity and DNA content, oncogenes and tumor suppressor genes [3]. Recent attention has been directed singularly at molecular classifications of breast cancer. While molecular and genetic testing is very elegant, prognostic and predictive, it is expensive and not yet widely available [4]. The Immunohistochemistry (IHC) classification provides both therapeutic and prognostic information. These assays have the advantage of allowing only tumor cells to be assessed for receptor status. They can be conducted relatively inexpensively on routinely processed tissue sections with no need for specialized equipment [4].

Oestrogen and Progesterone receptor scoring by Immunohistochemistry ER/PR (Quick score method) [5] was as per **Table – 1**. "Any nuclear immune staining for ER should be considered as a positive result" according to national Institute of Health (NIH) consensus 2000 [2]. The other scoring systems are J-score [6], H – score, Allred score [7] and advocated by Yaziji, et al. [8].

HER-2/Neu Scoring (ASCO guidelines) [8]

Nature of Staining Score

- No staining or membranous staining in 0 <10% of cells
- Incomplete membranous staining in 1 >10% of cells
- Complete membranous staining in >10% of 2 Cells of weak to moderate intensity
- Complete membranous staining in >10% cells 3 of strong intensity

Triple Negative breast cancer: Triple negative breast cancer, defined as that with negative expression of Oestrogen and

Progesterone receptors and HER-2 accounted for 10-17% of all breast carcinomas [9]. Women with triple-negative breast cancer were generally postmenopausal, with adverse pathological characteristics of high histological grade and frequent nodal metastasis [10, 11].

Table - 1: Oestrogen and Progesterone receptor scoring by Immunohistochemistry ER/PR (Quick score method) [5].

ER/PR	% Positive Cells	Score	Staining Intensity	Score	Total Score
	NIL	0	-	-	0
	<1	1	MILD	1	2
	1-10	2	MODERATE	2	3
	11-33	3	INTENSE	3	4
	34-66	4			5
	67-100	5			6
					7
					8

Aims and objectives

- To assess the ER/PR status and HER -2 status of breast carcinoma.
- To correlate the ER/PR and HER-2 status with modified Bloom- Richardson histological grading and clinico pathological parameters.

Materials and methods

The study was conducted for 2 years, from August 2013 to July 2015 at Chalmeda Anand Rao Institute of Medical Sciences, Karimnagar. The study included 75 patients with breast cancers to study the Immunohistochemistry for the detection of ER/PR and HER-2/neu status and correlation with histopathological grading of breast cancers. The following parameters at the time of presentation were noted from the Department of Pathology, Age, sex, menstrual status, Mode of presentation e.g. lump/ nipple discharge, procedure e.g. biopsy or mastectomy, tumor size on gross examination, histologic subtype of breast cancer and axillary nodal status.

The hematoxylin and eosin (H&E) sections of the cases were retrieved from the records and screened for confirmation of diagnosis and selection of representative tumor paraffin blocks. The representative neoplastic tissue blocks (paraffin embedded) were cut at 3.0µ on Poly-L -Lysine coated slides. One of these sections was routinely stained with H&E. The histologic grading of tumor was done on H&E stained sections according to Modified Bloom and Richardson grading.

Results

In the present study, age ranged from 21-85 years and the mean age ± SD was 54.04 ± 12.3 years. Majority, 44 cases (58.6%) belonged to 41-60 years followed by 12 (17.3%) 31-40 years, 9 (12%) 61-70 years, 4 (5.3%) 21-30 years, 4 (5.3%) 71-80 years and 1(1.3%) > 80 years most of the patients were postmenopausal 40 (53.3%), 18 (24.0%) were perimenopausal and 17 (22.7%) were premenopausal (**Table - 5**). 3 cases (4%) had family history of breast cancer. 5 cases had past history of carcinoma breast, 8cases (10%) had history of exogenous oestrogen intake. All cases presented with breast lump which was the commonest symptom in 63cases (84%), followed by breast lump with pain in 4 cases (5.3%), majority 53 specimens (70.7%) were modified radical mastectomy, 11(14.7%) were lumpectomy, 7(9.3%) simple mastectomy, 3(4%) biopsies and 1(1.3%) quadrantectomy. On gross examination, 49 cases (65.3%) measured >5 cms, followed by 21 cases (28%) between 2.0-5.0 cms and 5 cases (6.7%) ≤ 2 cms.

Table - 2: Histologic (MBR) grade.

Histologic (MBR) grade	Number of patients (75)	Percentage
Grade I	24	32.0
Grade II	27	36.0
Grade III	24	32.0

Table - 3: Final histopathological diagnosis.

Final Diagnosis	No. of Patients	%
IDC (NOS)	69	92.0
Lobular Carcinoma	2	2.7
Papillary Carcinoma	1	1.3
Mucinous Carcinoma	1	1.3
Medullary Carcinoma	1	1.3
Apocrine carcinoma	1	1.3

Table - 4: ER, PR and HER-2 status.

ER,PR and HER-2	No. of patients (75)	%
ER		
Positive	57	76
Negative	18	24.0
PR		
Positive	44	58.7
Negative	31	41.3
HER-2		
Positive	33	44.0
Negative	42	56.0

Histologic grading showed 27(36.0%) of cases to be grade-II and grade-I and grade III included 24(32%) cases each (**Table - 2**).

In our study the predominant histologic subtype was Infiltrating ductal carcinoma (NOS), 2 cases were lobular carcinomas and 1case each of papillary, mucinous, medullary and apocrine carcinomas (**Table - 3**).

39 cases (52%) had nodal metastasis, 15(20%) were reactive and 1(1.3%) had epithelioid cell granulomas. In case of biopsy and lumpectomy, lymph nodes were not available for study. 12(16%) cribriform, 10(13.3%) comedo, 8 (10.7%) mixed and 4 (5.3%) had solid in-situ component. Paget's disease was seen in 11 cases (14.75%).

57 (76%) tumors expressed ER, 44(58.7%) tumors expressed PR and 33(44%) expressed HER-2/neu (**Table - 4**).

Of 75 cases, 30 (50%) were ER/PR+ HER-2-, 14(23.3%) were ER/PR- HER-2+, 13(21.7%) were ER/PR+ HER-2+ and remaining 3(5%) were triple negative (**Table - 5**).

The most common histologic subtype in which ER, PR and HER-2 positivity were noted was IDC (NOS). 52/57 (91.2%) of ER positive cases, 42/44 (95.5%) of PR positive cases, and 31/33 (93.9%) of HER-2 positive cases were IDC (NOS). There were 2 cases of lobular carcinoma, both were positive for ER and another was positive for both ER & PR. 1 papillary carcinoma studied was positive for both ER & PR. 1 mucinous carcinoma diagnosed in the study was positive for only ER. 1 medullary carcinoma was encountered in this study which was positive for HER-2. 1 apocrine carcinoma found in this study was positive for both ER & HER-2. ER positivity was noted in 27 cases (47.4%) of postmenopausal women (Table 6). ER positivity was most common with tubule formation, nuclear pleomorphism, and mitotic grade of score-2. Histologic grading in correlation with ER positivity was found to be statistically significant ($p < 0.001$). 23(40.4%) of grade I, 24 (42%) of grade II and 10 (17.5%) of grade III tumors were positive for ER. PR positivity was noted in 22 cases (50%) of postmenopausal women. PR positivity was most common with tubule formation, nuclear pleomorphism and mitotic grade of score-2. Histologic grading in correlation with PR positivity was found to be statistically significant ($p<0.001$). 22(50%) of grade I, 18 (40.9%) of grade II and 4 (9.1%) of grade III tumors were positive for PR.

Out of 33 positive cases of HER-2/neu, 20 cases (60.6%) were in postmenopausal

women, followed by 7 cases (21.2%) of perimenopausal and 6 cases (18.2%) of premenopausal women. HER-2 positivity was most common with tubule formation and mitotic rate of score-3 and nuclear pleomorphism of scroe-2. Histologic grading in correlation with HER-2 positivity was found to be statistically significant ($p<0.001$). 16(48.5%) of grade III, 14(42.4%) of grade II and 3(9.1%) of grade I tumors were positive for HER-2. As we have considered score-2+ & 3+ both as HER-2 positive cases, the number of cases in the post-menopausal age group appears more in our study.

Table - 5: Immunohistochemical subtypes.

ER/PR and HER2	Number of patients (60)	Percentage	95%CI
ER/PR+ HER2-	30	50.0	37.74-62.28
ER/PR+ HER2+	13	21.7	13.12-33.62
ER/PR- HER2-	3	5.0	1.71-13.70
ER/PR- HER2+	14	23.3	14.44-35.44

Table – 6: Relationship between histologic subtypes and ER, PR and HER-2 positivity.

Histologic Subtype	ER+ (n=57)	PR+ (n=44)	HER-2+ (n=33)
IDC (NOS)	52 (91.2%)	42 (95.5%)	31 (93.9%)
Lobular Carcinoma	2 (3.5%)	1 (2.3%)	0 (0%)
Papillary Carcinoma	1 (1.8%)	1 (2.3%)	0 (0%)
Mucinous Carcinoma	1 (1.8%)	0 (0%)	0 (0%)
Medullary Carcinoma	0 (0%)	0 (0%)	1 (3%)
Apocrine carcinoma	1 (1.8%)	0 (0%)	1 (3%)

Subjects with ER/PR+ HER-2- were 30 in number; younger compared to the other subtypes, with more number of stage II cancer. Histologic grades were well correlated with the immunohistochemical subtypes ($p <0.001$). 2(14.3%) of grade II and 12 (85.7%) of grade III tumors were ER/PR-, HER-2+. Most of them were larger than 5cms, 10 (33.3%) showed LVI. Majority 93.3%were IDC (NOS). There were 13 triple positive cases most were larger than 5cms, and all of them were IDC (NOS). The triple negative cases were only 3 and all were larger than 5cms with IDC (NOS) type. There were 14 ER/PR, HER-2+ cases (**Table – 7, Figure – 1A, 1B**).

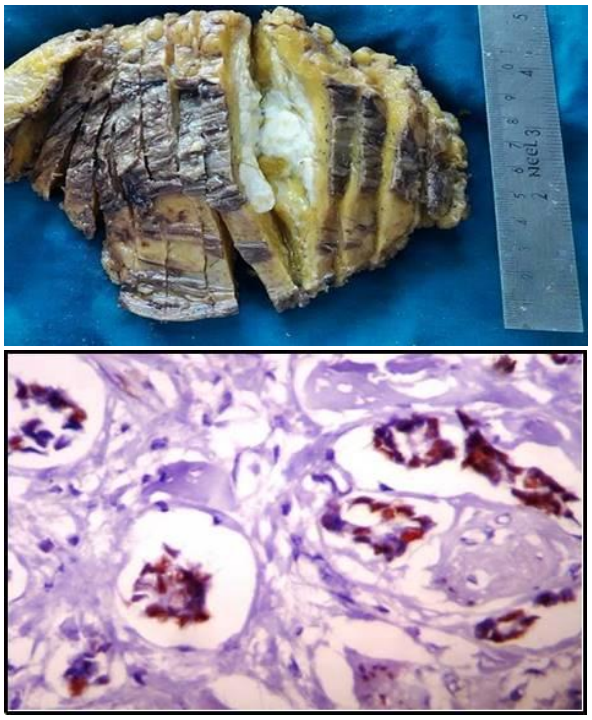
Discussion

As Breast cancer being the most common cancer among women in India and in many regions of the world, constant research on prognostic and predictive markers of breast carcinoma is going on. There is one in eight chance of developing breast cancer in women who lives to the age of 90 years. Classic variables such as histologic type and grade, tumor size, lymph node status, status of hormone receptors – Oestrogen receptor (ER) and Progesterone receptor (PR) of the tumor, and more recently HER-2/neu status influence the prognosis and management.

The current trend in analyzing the clinical outcome of the patient with breast cancer is to examine predictive and prognostic factors related to the patient and the tumor. Predictive factor is related to degree to which the patient could respond to specific therapy, while prognostic factor is related to metastatic potential of the tumor. With advancement in science and technology, new molecular methods are giving insight into biology of breast cancer and opening avenues for developing therapeutic strategies and predict the outcome. So, we took to study these important prognostic markers

and correlate with the histological grading of breast cancers. Our study is a prospective study which included 75 cases of breast cancers which were proven histopathologically during the period of August 2014 to July 2015.

Figure - 1A, 1B: IDC (NOS) Gross specimen and Grade I IDC (NOS) with predominant tubular pattern (x400 H & E).



ER, PR, HER-2 Vs age and menstrual status

Onitilo AA, et al. [12] and Huang JH, et al. [13] have shown that subjects with ER/PR+, HER-2- subtypes were more likely to be older and postmenopausal when compared to premenopausal ones. Our study also shows similar results (**Table - 8**).

Present study data are consistent with those of other published studies like those of Huang JH, et al. [13] and Onitilo AA, et al. [12] (**Table - 8**); in that ER and or PR expression is generally correlated inversely with HER-2 over expression. However, a substantial number of HER-2+ tumors still expressed ER and or PR. In our study they constitute 21.7%. Lal P, et al. [14], Ayadi L, et al. [15] and other studies in literature demonstrated high ER, PR positivity with IDC (NOS), invasive lobular carcinomas and mucinous carcinomas. But medullary carcinomas evoked controversial results. When HER-2status was analyzed according to histologic features, HER-2 positivity was limited to IDC (NOS). In our study 1 each of medullary and apocrine carcinomas were positive for HER-2, 1 papillary carcinoma was positive for both ER & PR and negative for HER-2 (**Table - 9**).

Onitilo AA, et al. [12], Ayadi L, et al. [15], Huang JH, et al. [13], Perio G, et al. [16] and Lal P , et al. [14] have shown that well differentiated tumors express hormone receptors with decreased expression of HER-2.In our study 63.3% of Grade-I tumors expressed both ER, PR with negativeHER-2 expression while only 6.7% of grade III were this subtype. None of the grade IER/PR- tumors expressed. HER-2. 85.7% of Grade-III tumors expressed HER-2 with negative ER/PR expression, indicating that poorly differentiated tumors have less hormone receptors with increased HER-2 expression. Therefore, over expression ofHER-2 is inversely related to ER/PR status. Stierer M, et al. [10] correlated individual characteristics like tubule formation, nuclear pleomorphism and mitotic rate with steroid receptor status. Our findings were similar to other studies as in Lal P, et al. [14], Nadji M, et al. and Peiro G, et al. [16]. 64.3% tumors of ER/PR-, HER-2+, subgroups were in stage III and 20% of tumors of ER/PR+, HER-2+ subgroup were in stage III. This reflects the higher incidence of metastasis and aggressive biologic behavior with HER-2 over expression (**Table - 11**).

Conclusion

Prognosis and management of breast cancer are influenced by classic variables such as histologic type and grade, tumor size, lymph node status, status of hormone receptors- ER, PR and more recently, HER-2 status. The interrelationship between ER, PR and HER-2 has come to have an important role in the

management of breast cancer. Patients with breast carcinoma over expressing HER-2 do not respond to tamoxifen therapy. Recently anti-HER-2antibodies (Herceptin) have been shown to be effective against HER-2 over expressing breast carcinomas.

Table - 7: Clinicopathological correlation with immunohistochemical subtypes.

Clinical variables	**ER/PR+ & HER2neu-**	**ER/PR+ & HER2neu+**	**ER/PR-HER2neu-**	**ER/PR-& HER2neu+**	**p value**
Age (Min-Max) (yrs)	28-70	35-84	52-65	35-75	
Age in years	49.97±12.53	49.46±15.13	58.33±6.51	54.14±11.39	0.534
Duration in months	9.10±6.42	10.23±4.97	5.67±1.16	7.93±5.48	0.568
Tumor stage					
I	2(6.7%)	0	0	0	0.044+
II	14(46.7%)	3(23.1%)	0	2(14.3%)	
III	6(20.0%)	4(30.8%)	1(33.3%)	9(64.3%)	
Tumor Size					
<2 cms	3(10.0%)	1(7.7%)	0 (0%)	1 (7.1%)	0.422
2-5 cms	11(36.7%)	1(7.7%)	0 (0%)	4 (28.6%)	
>5 cms	16(53.3%)	11(84.6%)	3 (100%)	9 (64.3%)	
Lympho vascular Invasion					
Present	10(33.3%)	6(46.2%)	1 (33.3%)	12 (85.7%)	0.008**
Absent	20(66.7%)	7(53.8%)	2 (66.7%)	2 (14.3%)	
Cancer type					
IDC (NOS)	28(93.3%)	13(100.0%)	3 (100%)	13 (92.9%)	0.881
Lobular Carcinoma	1(3.3%)	0	0 (0%)	0 (0%)	
Papillary Carcinoma	1(3.3%)	0	0 (0%)	0 (0%)	
Mucinous Carcinoma	0	0	0 (0%)	0 (0%)	
Medullary Carcinoma	0	0	0 (0%)	1 (7.1%)	
Apocrine carcinoma	0	0	0 (0%)	0 (0%)	
Histologic Grade					
Grade I	19(63.3%)	3(23.1%)	1(33.3%)	0	<0.001**
Grade II	9(30.0%)	8(61.5%)	0	2(14.3%)	
Grade III	2(6.7%)	2(15.4%)	2(66.7%)	12(85.7%)	

Table – 8: Association of ER, PR expression with HER-2 status.

Immunohistochemical subtypes	**Onitilo AA, et al. [12] %**	**Huang J H, et al**. [13] **%**	**Present study %**
ER/PR+, HER2-	68.9	66.4	50.0
ER/PR+, HER2+	10.2	30.9	21.7
ER/PR-, HER2-	13.4	13.8	5.0
ER/PR-, HER2+	7.5	45.6	23.3

In this study an attempt was made to understand the correlation of ER, PR and HER-2 status with histopathological grading and clinicopathological parameters. In conclusion, ER, PR and HER-2 status correlates well with histopathological grading and other clinico-pathological parameters. Higher grade is associated with HER-2 positivity and ER/PR negativity, larger tumor size, lympho vascular invasion, lymph node metastasis, and higher clinical stage. Hence, immunohistochemical assessment of ER, PR

and HER-2 status should be incorporated as a routine investigation. This along with histopathological grading and staging will guide the clinicians to make correct choice of treatment protocols.

Table - 9: ER, PR and HER-2, status in different tumor types.

	Authors								
Histologic Subtype	**Lal P, et al. (%)[14]**			**Ayadi L, et al. (%)[15]**			**Present study (%)**		
	ER	**PR**	**HER-2**	**ER**	**PR**	**HER-2**	**ER**	**PR**	**HER-2**
IDC (NOS)	71.58	47.38	17.54	61.1	53.8	16.8	73.36	60.86	44.92
Lobular Carcinoma	93.3	60.2	0.8	50	50	16.7	100	50	0
Mucinous Carcinoma	100	70	0	60	60	0	100	0	0
Medullary Carcinoma	-	-	-	0	0	0	0	0	100
Papillary Carcinoma	-	-	-	-	-	-	100	100	0
Apocrine Carcinoma	-	-	-	-	-	-	100	0	100
Metaplastic Carcinoma	0	0	0	-	-	-	-	-	-
Adenoid cystic Ca.	0	0	0	-	-	-	-	-	-
Endocrine Carcinoma	-	-	-	66.7	33.3	33.3	-	-	-
Oncocytic Carcinoma	-	-	-	0	0	100	-	-	-

Table - 10: Comparison of Immunohistochemical subtypes with Histologic grading.

Immuno-histochemical Subtypes (%)	**Onitilo AA, et al. [12]**			**Present study**			**P Value**
	Histologic Grades						
	Grade I	**Grade II**	**Grade III**	**Grade -I**	**Grade II**	**Grade III**	<0.001
ER/PR+, HER2-	28.9	44.9	21.5	63.3	30.0	6.1	
ER/PR+, HER2+	6.0	41.4	49.1	23.1	61.5	15.4	
ER/PR-, HER2-	4.0	12.5	76.3	33.3	0	66.7	
ER/PR-, HER2+	1.2	20.0	77.7	0	14.3	85.7	

Table - 11: ER, PR, HER-2 and histologic grades.

Histologic Grade		**Ayadi L, et al.**		**p Value**
	HER-2 +(%)	**ER+(%)**	**PR + (%)**	
Grade I & II	14.8	72.2	61.4	< 0.000
Grade III	27.5	22.5	27.5	

Huang JH, et al. [13]			
Histologic Grade	**Grade I & II**	**Grade III**	**p Value**
ER/PR	**HER-2 +ve(%)**	**HER-2 +ve (%)**	
ER-PR-	31.0	28.5	< 0.001
ER-PR+	0	46.2	
ER+ PR-	7.4	18.9	
ER+ PR+	2.7	12.9	

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