International Journal of Clinical and Diagnostic Pathology

ISSN (P): 2617-7226
ISSN (E): 2617-7234
www.patholjournal.com
2021; 4(4): 21-25
Received: 16-08-2021
Accepted: 18-09-2021

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Correlation of immunohistochemistry markers in breast carcinoma with biological characteristics and tumor grade as histopathological prognostic parameters

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DOI: https://doi.org/10.33545/pathol.2021.v4.i4a.418

Abstract
Background: Prognosis of breast cancer and success of therapeutic interventions largely rely on clinico-pathologic and biological characteristics of tumor and vary due to the heterogenous nature of breast cancers. This study was intended to find correlation between the expression of Estrogen receptor (ER), Progesterone receptor (PR) and Human Epidermal Growth Factor Receptor-2 (HER-2/neu) with histological grade in case of carcinoma of breast.
Material and Method: This study was conducted on 56 histologically confirmed specimens of breast carcinoma in Department of Pathology, Surat Municipal Institute of Medical Education and Research.
Results: Maximum number of cases of breast carcinoma were found in 41-50 years aged females. Invasive ductal carcinoma-not otherwise specified (IDC-NOS) (82.14%) was most common histological type. Most of the carcinoma showed grade II (62.5%). ER, PR and HER-2/neu positivity was 50%, 42.86% and 42.86% respectively.
Conclusion: Evaluation of ER, PR and HER-2/neu status and tumor grade should be incorporated routinely as a part of histopathological reports in all cases of breast carcinoma as they not only help in histopathologic assessment of prognosis, but also help in deciding the management thereby improving overall survival.

Keywords: carcinoma of breast, estrogen receptor, progesterone receptor, human epidermal growth factor receptor-2

Introduction
There is a need to understand the initiation and progression of breast cancer on hormonal, cellular and molecular basis to design the targeted therapy as early diagnosis and potential curability with proper treatment of early breast lesions can lead to improvement in mortality due to breast cancer.
Several histopathological features have prognostic significance in cases of carcinoma breast including histologic subtype, grade, lymph node status, estrogen receptor (ER) and progesterone receptor (PR) status, human epidermal growth factor and its receptors [1]. The presence of hormone receptors (ER and PR) in the tumor tissue has been found to correlate well with the response to hormone therapy and chemotherapy. Human epidermal growth factor receptor-2. HER-2/neu overexpression indicates low response to tamoxifen and decreased survival according to several studies [2, 3]. The ER and HER2 signalling pathways are the dominant drivers of cell proliferation and survival in majority of breast cancers. Targeting these pathways provides the most effective therapy in appropriately selected patients.
Conventional histopathological grading along with hormone receptor status analysis has become a prerequisite for management and prognosis of breast carcinomas in this era. Present study was intended to find correlation between the expression of ER, PR and HER-2/neu with biological characteristics (age and lymph node status) and histological grade in cases of carcinoma breast. These parameters can be useful not only in diagnosis and assessment of prognosis, but also in deciding management of breast carcinoma, thereby improving overall survival [1].

Material and Method

The present study was done in 56 confirmed cases of breast carcinoma specimen in the Department of Pathology at a tertiary care teaching hospital, Surat Municipal Institute of Medical Education and Research.

Inclusion Criteria: Modified Radical Mastectomy Specimen of Breast Carcinoma in female

Exclusion Criteria

1. Male Breast
2. True cut Biopsy

Data collection

Patient's clinical data were collected from histopathological requisition forms and hospital records. This study was done in duration of one year. All the histopathological slides were retrieved and representative slides were evaluated for patient's age, typing and grading of the tumor. The representative blocks were selected and ER, PR, Her2neu IHC markers were done. Positive and negative controls were included during staining. Correlation of ER, PR, Her2neu with biological characteristics and histological grade factors was done.

Result

Table 1: Correlation of ER, PR and HER-2/neu expression with age (n=56)

Age Group (Year)		ER status		PR status		HER2/neu over expression	
		Positive	Negative	Positive	Negative	Positive	Negative
<50	29(51.79%)	12(41.38%)	17(58.62%)	10(34.48%)	19(65.52%)	12(41.38%)	17(58.62%)
>50	27(48.21%)	16(59.26%)	11(40.74%)	14(51.85%)	13(48.15%)	12(44.38%)	15(55.56%)
Total	56 (100%)	28(50%)	28(50%)	24(42.86%)	32(57.14%)	24(42.86%)	32(57.14%)

Table 2: Correlation of ER, PR and HER-2/neu expression with Tumor size

Tumor size (cm)		ER status		PR status		HER-2/neu over expression	
		Positive	Negative	Positive	Negative	Positive	Negative
T1 (<2 cm)	3(5.36%)	2(66.67%)	1(33.33%)	2(66.67%)	1(33.33%)	01(33.33%)	02(66.67%)
T2 (2-5cm)	41(73.21%)	21(51.22%)	20(48.78%)	18(43.90%)	23(56.10%)	16(39.02%)	25(60.98%)
T3(>5cm)	12(21.43%)	5(41.67%)	7(58.33%)	4(33.33%)	8(66.67%)	07(58.33%)	05(41.67%)
Total	56(100%)	28(50%)	28(50%)	24(42.86%)	32(57.14%)	24(42.86%)	32(57.14%)

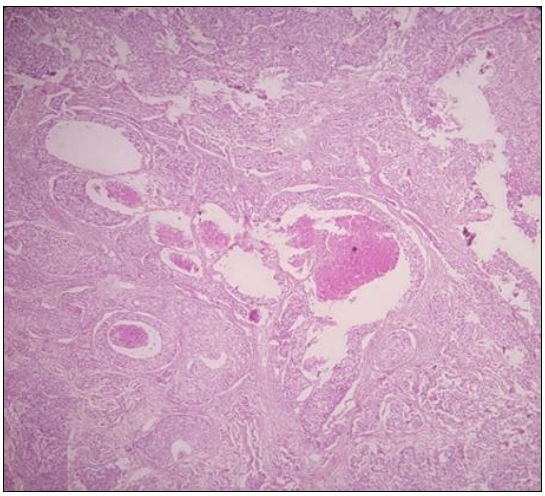

Fig 1: Ductal Carcinoma NOS Type H & E stain

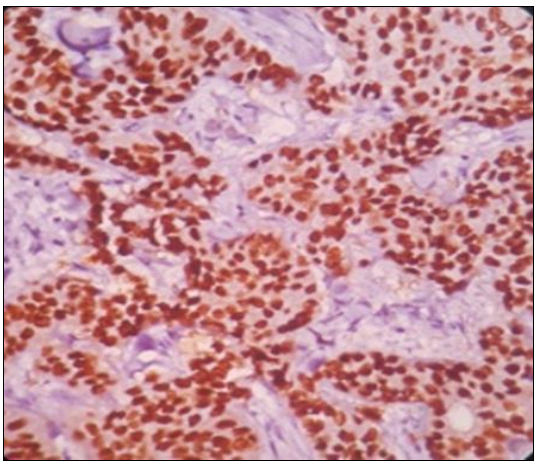

Fig 3: Nuclear Positivity for Estrogen Receptor in Ductal Carcinoma (IHC, 40x)

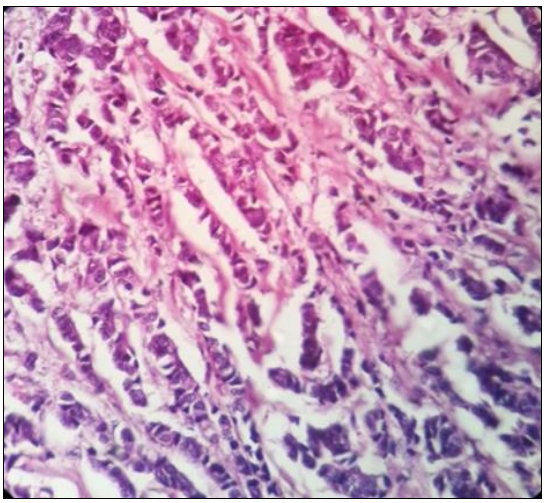

Fig 2: Infiltrative lobular carcinoma with ductal carcinoma

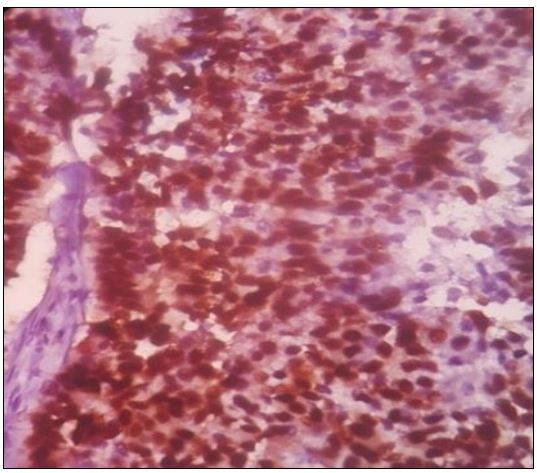

Fig 4: Nuclear positivity for progesterone receptor positivity (IHC, 40x)

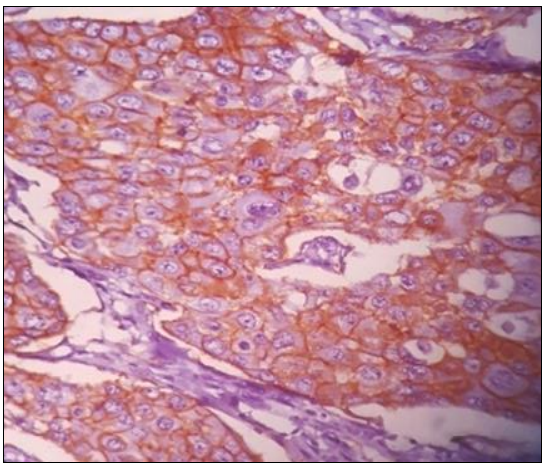

Fig 5: Ductal Carcinoma, showing membrane positivity for HER-2/neu

Table 3: Status of hormone receptor in breast cancer

Hormone receptor		Cases	Percent %
ER/PR	ER+/PR+	23	41.07
	ER+/PR-	5	8.93
	ER-/PR+	1	1.79
	ER-/PR-	27	48.21
Total		56	100

Table 4: Correlation of ER, PR and HER-2/neu expression with involvement of Axillary Lymph node

	Axillary lymph node involvement					
	Negative		1-3 Positive		>3 Positive	
ER Positive	09	(52.94%)	09	50.00%	10	47.62%
PR Positive	08	47.06%	08	44.44%	08	38.10%
HER-2/neu Positive	05	29.41%	08	44.44%	12	57.14%
Total=56(100%)	22	30.36%	18	32.14%	21	37.50%

Table 5: Correlation of ER, PR and HER-2/neu expression with histological type

Tumor type	Total	ER+	PR+	HER-2/neu+
IDC – NOS	46(82.14%)	22(47.83%)	18(39.13%)	20(43.47%)
Medullary Carcinoma	3(5.35%)	0	0	2(66.67%)
IDC+ Mucinous change	2(3.57%)	2(100%)	2(100%)	1(50%)
IDC + ILC	2 (3.57%)	2(100%)	2(100%)	0
Papillary Carcinoma + IDC	1(1.79%)	1(100%)	1(100%)	0
Tubular ca + Ductal Ca	1(1.79%)	1(100%)	1(100%)	0
IDC + Paget's Disease	1(1.79%)	0	0	1(100%)
Total	56 (100%)	28(50%)	24(42.86%)	24(42.86%)

Table 6: Association of HER-2/neu with ER and PR expression

HER-2/neu status		Estrogen receptor expression				Progesterone receptor expression			
		Positive		Negative		Positive		Negative	
Negative	32	20	62.%	12	37.%	18	56.25%	14	43.75%
Positive	24	8	33.33%	16	66.67%	6	25%	18	75%
Total	56 (100%)	28	50%	28	50%	24	42.86	32	57.14%

Table 7: Correlation of ER, PR and HER-2/neu expression with Tumor grade

Tumor Grade		ER status		PR status		HER-2/neu overexpression	
		Positive	Negative	Positive	Negative	Positive	Negative
Grade I	8(14.29%)	7(87.5%)	1(12.5%)	7(87.5%)	1(12.5%)	3(37.5%)	5(62.5%)
Grade II	35(62.5%)	19(54.29%)	16(45.71%)	16(45.71%)	19(54.29%)	15(42.86%)	20(57.14%)
Grade III	13(23.21%)	2(15.38%)	11(84.62%)	1(7.69%)	12(92.31%)	6(46.15%)	7(53.85%)
Total	56(100%)	28 (50%)	28(50%)	24(42.86%)	32(57.14%)	24(42.86%)	32(57.14%)

56 confirmed cases in female breast carcinoma have been included in the study. The maximum number of cases were found in 41-50 (37.5%) followed by 51-60(28.57%). Mean age was 50+/- 10 years. Estrogen receptor was found to be positive in 50% of cases. On comparing receptor positivity with histological grade, the positivity of ER decreased with increasing grade. Progesterone receptor status was found to be positive in 42.86% of cases. With increasing the histological grade, the positivity of PR was reported to decrease. The correlation between ER and PR positivity with histological grade was statistically significant (p value <0.05). HER-2/neu positivity was found in 42.86% of cases. HER-2/neu positivity was found to increase along with histological grade. However, no significant statistical correlation was found between HER-2/neu status with histological grade (p value >0.05). In present study showed ER and PR expression was seen to be decreased with increased number of positive lymph nodes. HER-2/neu overexpression was increased as number of positive lymph nodes increased. In present study, the most common histological type was Invasive Ductal carcinoma, NOS type, 46 cases (82.14%). % ER & PR and HER-2/*neu* positivity was not affected by histological type of tumor.

There was a statistically significant association between HER-2/neu status and ER expression and PR expression (p value <0.05). ER and PR expression correlated inversely with HER-2/neu over-expression (ER 33.33% v/s 62.5% and PR 25% v/s 53.12%).ER and PR expression were decreased significantly in HER-2/neu positive compared with HER-2/neu negative tumors (ER 66.67 v/s 37.5%; PR 75% v/s 46.88%). In this study, HER-2/neu protein overexpression was associated with a statistically significant higher rate of ER- and PR- negative status. Triple negative expression of ER, PR and HER-2/neu was found in 10 cases (17.86%) out of which 4 cases (46.15%) were found to be in Grade 3(46.15%).

Discussion

Prognosis and management of breast cancer are very much influenced by prognostic factors such as histological type

and grade, lymph node involvement, hormone receptor status and HER-2/neu overexpression. In our study, the maximum number of 21 cases (37.5%) was found in the age group of 51-60 years. 16 cases (28.57%) were found in age group of 41-50 years. Our study correlated well with the study of Nabi *et al.* [4] having maximum number of cases found in the age group of 51-60 years (33.81%). Bhagat *et al.* [6], Verma *et al.* [5] and Singh *et al.* reported maximum number of cases as 41.37%, 43.75% and 48.15% respectively in the age group of 41-50 years. The most common histological type of breast carcinoma diagnosed in the present study was invasive ductal carcinoma (not otherwise specified) in 46 cases (82.14%). It was also reported as the predominant histological type in the studies by Bhagat *et al.* [6], Thiygarajan *et al.* [10], Tiwari *et al.* [8] and Verma *et al.* [5] as 94.82%, 84.3%, 81.17% and 87.5% respectively.

In our study, majority of the cases presented with grade II tumor (62.5%), followed by grade III (23.21%) while least number of the patients belonged to grade I(14.29%). Grade II had also been observed as the most common grade by the studies of Nisa *et al.* [11] (55.33%), Ahmed *et al.* [7] (55.2%), Bhagat *et al.* [6] (43.1%), Geethmala *et al.* [12] (54.0%) and Tiwari *et al.* [8] (68.7%).

We reported lymph node positivity as 69.64%. Our findings correlated well with the studies of Bhagat *et al.* [6] and Thiyagarajan *et al.* [10] with lymph node involvement seen in 53.44% and 53.3% of the cases respectively. However, Ahmed *et al.* [7], Nisa *et al.* [11] and Siadati *et al.* [13] had a higher incidence of lymph node involvement i.e.75.3%, 71.3 and 85.67% respectively.

We observed that positivity for estrogen receptor decreased as the grades increased. The findings show close resemblance with various studies conducted by Nisa *et al.* [11], Nikhra *et al.* [14], Gore *et al.* [15], Verma *et al.* [5] and Singh *et al.* [9] Positivity for PR also decreased as the grades increased in our study. Similar findings were observed in studies conducted by Nisa *et al.* [11], Verma *et al.* [5], Gore *et al.* [15], Nikhra *et al.* [14] and Singh *et al.* [9]. On comparing HER-2/neu positivity with histological grade, we found that the positivity for HER-2/neu increased with increasing histological grade. However, no statistical significance was observed (p> 0.05) on comparing grade and HER-2/neu overexpression. Similar findings were reported by Nisa *et al.* [11], Bhagat *et al.* [6] and Gore *et al.* [15].

Overall, ER positivity was observed in 50% cases while PR positivity was seen in 42.86%. HER-2/neu overexpression was seen in 42.86% cases in present study. Similar findings were obtained in studies of Nisa *et al.* [11], Verma *et al.* [5], Nikhra *et al.* (14) and Singh *et al.* [9].

On comparison of HER-2/neu with ER and PR positivity, it was observed that in HER-2/neu positive cases, ER was positive in 8 cases (33.33%) and PR was positive in 6 cases (25%). In HER-2/neu negative cases, ER was positive in 23 cases (62.5%) and PR was positive in 18 cases (56.25%) of the cases. Similar strong inverse relationship was demonstrated by study conducted by Almsari *et al.* [16], Huang *et al.* [17] and Yadav *et al.* [18] also reported inverse relationship between ER and PR positivity with HER-2/neu overexpression.

HER-2 positive breast cancers are ER-positive but they generally have lower ER level. These tumors have high proliferation rates, more aneuploidy and are associated with poorer patient prognosis. The poor outcomes dramatically improved with appropriate chemotherapy combined with the HER2-targeting drugs [19].

We observed 17.86% triple negative cases in our study. The association between triple negative receptor status of tumors and histological grading was found to be statistically significant (p<0.05). These findings are in correlation with those observed in studies of Geethmala *et al.* [12], Verma *et al.* (5) and Singh *et al.* [5] where triple negative cases were found to be 20.0%, 28.12% and 30.0% respectively and the pattern of grade-wise percentage of triple negative cases increased with severity of histological grade, which indicates poor prognosis [20].

The expression of ER, PR and HER-2 and tumor grade are valuable for subtyping of breast carcinoma for prognostic evaluation. These IHC markers are simple, inexpensive, east to interpret, reliable, reproducible and readily available.

Limitation of present study

Fluorescence in Situ Hybridization (FISH) determines the Her-2/neu true positivity. Equivocal cases of Her2neu were not confirmed by FISH due to unavailability of the same at our institute & financial constraints of patients.

Conclusion

A significant correlation with histological grade was observed with expression of ER and PR expression, but not seen with HER-2/neu expression. The association between triple negative receptor status of tumor and histological grading was found to be significant.

Hence, immunohistochemical assessment of these biomarkers along with grading of breast carcinoma improves the prognostic accuracy of histopathological assessment. This reflects the importance of these IHC markers and tumor grade to be included in routine histopathology work-up to provide valuable information for the best therapeutic intervention in breast carcinoma patients, thereby improving overall survival.

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