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Original Research Article

A Correlation Study between Clinical, Histomorphological Features and Estrogen and Progesterone Receptors and Her2/Neu Expression in Carcinoma Breast

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Abstract

Background: Breast carcinoma is one of the leading causes of malignancy in females.Assessment of Estrogen Receptors (ER)/ Progesterone receptors (PR) and HER2/neu expression in breast cancer is mandatory in clinicalpractice. Immunohistochemistry (IHC) for assessing hormonal receptor status is easier, safer, and hasbetter ability to predict response to adjuvant endocrine therapy. HER2/neu overexpression is shown to have important prognostic and predictive value. The best approach to the use of immunohistochemical markers is to couple them with standard Haematoxylin & Eosin (H&E) based histology and to use panel of markers. *Objectives:* To assess the ER, PR & HER2/neu status and correlate with histological grade and other clinicopathological parameters. *Materials and methods:* Fifty cases of breast carcinoma were taken forthe study. H&E sections diagnosed as carcinoma were assessed for histological type and grade. One dedicated block from tumour, not fixed for more than 24 hours in 10% formalin was used for ER/PR and HER2/neu receptor evaluation by IHC. Statistical analysis was done with SPSS software, using chi-square test. *Results:* A Total of 50 carcinoma breast cases were subjected to IHC. Twenty four cases (48.0%) were of histologic grade-II, 17 cases (34%) of grade III and nine cases (18%) were of grade I. ER, PR and HER2/neu status correlated significantly with histological grade. (p= 0.003). *Conclusion:* ER, PR & HER2/neu status correlates well with histopathological grading. Hence, immunohistochemical analysis should be incorporated in the routine histopathology reports and canbe of great value in deciding the treatment protocols.

Keywords: Breast Carcinoma; Immunohistochemistry; Er; Pr; Her2/ Neu; Histopathological Grading.

Introduction

Carcinoma of the breast remains one of the leading cancers in women today with an estimated life time risk of 13% [1].

Morphological classification of breast carcinomas divide these tumoursinto a number of subtypes. Approximately three fourth of mammary

carcinomaare invasive ductal or lobular type. Within these, and also among othermorphologic categories, tumours display marked heterogeneity in many of theirbiologic properties. One is the expression of steroid receptors in concert withthe oncogene ErbB2/Human epidermal growth factor receptors 2 (HER2). This has important clinical implications, such as selection of patients for endocrine therapy [2,3].

A large number of studies have correlated the presence or absence of tumour ER or PR with ultimate clinical outcomes. In addition to pathological grade and stage, we set out to evaluate tumour ER/PR status and HER2/neu expression by IHC as a prognostic and predictive factors in breast carcinoma [1].

Materials and Methods

A prospective study was conducted in a period of two years from June 2011 to May 2013, in theDepartment of Pathology, JJM Medical college, Davangere which included histologically proved carcinoma of breast (N = 50 cases) and excluded benign lesions of the breast and post chemotherapy biopsy specimens. Clinical details like age, sex, size of the tumor and lymphnode status were obtained from case sheets.

The specimens were thoroughly examined and clinical details were analysed. The specimen sent in formalin was sliced at one cm interval and fixedimmediately in 10% Neutral Buffer Formalin (NBF). One dedicated block from the tumor not fixed formore than 24 hours in formalin was used for IHC. Four µ thickness sectionswere cut and taken on Poly-L-Lysine coated slides and stained for evaluating ER, PR receptors and HER2/neu expression. And also sections were routinely stained with H & E and histological grading of tumour was done on H & E stained sections according to Modified Bloom – Richardson grading [4].

Procedure followed for IHC staining is according to guidelines given in Biogenex manual. Immunohistochemical stained sections were observed under lightmicroscopy and ER/PR nuclear staining was scored according to Allred scoremethod [5]. Allred score >2 was taken as positive for ER & PR. And HER2 membrane staining was interpreted according to ASCO/CAP guidelines [5]. Score 0, 1+ was taken as negative, 2+ as equivocal and 3+ as positive.

Statistical Analysis: Statistical analysis was done using spss software version 17. The relationship between clinicopathological parameters, IHC sub types and histopathological grading was done using chi-square test.

Results

Study design

An observational clinicopathological correlation study of 50 breast Cancer cases was undertaken to detect ER/PR and HER2/neu status by IHC and their correlation with histopathological grading ofbreast cancers and with clinical detailslike age, sex, size of the tumor and lymphnode status.

The age ranged from 15-69 years, the mean age being 45 years. Majority of the cases were in the age group of 31–50 years. Forty nine cases (98%) were of female patients and one case (2%) was of a 65 year old man.

The size of the tumour measured between 2.0-5.0 cmsin 38 cases (76%), followed by >5cms in nine cases (18%) and ≤2cms in two cases (4%). One was trucut biopsy measuring 1.5 cms in length.

Twenty four cases (48.0%) were of histologic grade-II followed by 17 cases (34%) and nine cases (18%) of histologic grade III and I respectively.

Out of 50 cases the predominant histological subtype was Infiltrating ductal carcinoma (IDC-NOS) accounting for 37 cases (74%), followed by four cases (8%) of medullary carcinoma, three cases (6%) of invasive lobular carcinoma (ILC), two cases (4%) of invasive micropapillary carcinoma and one case (2%) each of intracystic papillary carcinoma, IDC with papillary mucinous type, metaplastic carcinoma and secretory carcinoma.

Twenty four cases (48%) had nodal metastasis, five (10%) were negative for tumor deposits and in 21 cases (42%) nodes were not available for examination.

Immunohistochemical Profile

Twenty five (50%) cases expressed ER, 23(46%) cases expressed PR and 11(22%) cases expressed HER2/neu and two cases showed equivocal staining for HER2/neu which needs further evaluation by FISH.

Out of 50 cases, 19 (38%) were triple negative, 16(32%) were ER/PR+ HER-2-, six (12%) were ER/PR- HER-2+, five (10%) were triple positive, two cases (4%) were ER+/PR-HER2- and two cases (4%) were ER/PR+ HER2 equivocal.

In the present study, of 16 ER/PR+ HER-12 tumors (31.5%) measured 2 to 5 cms. Of the

19 triple negative tumours, 14 (36.8%) measured between 2 & 5 cms and five (55.6%) measured >5 cms. Eight cases of triple negative tumours followed by seven cases of ER/PR+ HER2-, four cases of ER/PR- HER2+ and three cases of triple positive tumors showed tumor deposits. Majority of the triple negative tumors (58.8 %) belonged to histological Grade 3. All ER/PR+ HER2- tumors belonged to histological grade 1 (66.7%) and grade 2 (41.6%). None of the grade 3 tumours were ER/PR+ HER2-. ER+, PR-, HER2- was seen in two cases which included one case of IDC papillary mucinous type of grade 2 and one case of IDC(NOS) of grade 3 with LN metastasis. Two cases showed ER/PR+ and HER2 equivocal which belonged to IDC (NOS) type of grade 1 and 2. (Table 1).

Discussion

Breast cancer is a heterogeneous disease composed of growing number of recognized biological subtypes. Prognostic indicators based on currently available clinical and histopathologic variables such as tumor size, tumor grade, lymph node status and hormone receptor status already exist and are used to predict a patient's clinical outcome in certain situations [6]. It is well known that ER, PR and HER-2 represent the most acceptable factors for predicting prognosis, response or resistance to treatment and the potential use of newer drugs [7]. Assessment of ER/PR and HER2 in breast cancer is mandatory in clinical practice [8].

Endocrine therapy (tamoxifen) is recommended for tumors expressing ER/PR. Patients with breast carcinoma overexpressing HER-2 do not respond to tamoxifen therapy. Recently anti HER-2 antibodies (Herceptin) have been shown to be effective against HER-2 overexpressing breast carcinomas.

So we undertook this study to evaluate for estrogen and progesterone receptors and her2/neu expression in various types of carcinoma breast cases and to correlate them with tumor type, histological grade and lymphnode status.

Frequency of immunohistochemical subtypes observed was consistent with the observation made by Satti MB et al. [8] Apart from these subtypes we also got two cases of ER+, PR-, HER2- which included one case of IDC papillary mucinous type of grade 2 and one case of IDC (NOS) of grade 3 with LN metastasis. Two cases showed ER/PR+

Table 1: Clinicopathological correlation with immunohistochemical subtypes

Clinical variables	ER/PR + & HER2/ neu-(n=16)	ER/PR + & HER2/ neu+(n=5)	ER/PR - & HER2/ neu-(n=19)	ER/PR - & HER2/ neu+(n=6)	p value
Age in Yrs	**26-69**	**30-67**	**15-67**	**34-55**	
Tumor Size (cms)					
• ≤2	2 (100%)	0	0	0	0.585
• 2-5	12 (31.5%)	3 (7.8%)	14 (36.8%)	5 (13.2%)	
• >5	2 (22.2%)	1 (11.1%)	5 (55.6%)	1 (11.1%)	
Histological subtype					
• IDC (NOS)	15(40.5%)	4 (10.8%)	11 (29.7%)	4 (10.8%)	0.053
• Medullary carcinoma	0	0	4 (100%)	0	
• ILC	0	1 (33.3%)	2 (66.7%)	0	
• Invasive micropapillary carcinoma	0	0	0	2 (100%)	
• Intracystic Papillary Carcinoma	1 (100%)	0	0	0	
• IDC with Papillary mucinous type	0	0	0	0	
• Secretory carcinoma	0	0	1(100%)	0	
• Metaplastic carcinoma	0	0	1 (100%)	0	
Histologic Grade					
• Grade I	6(66.7%)	1(11.1%)	1(11.1%)	0	0.003
• Grade II	10(41.6%)	3(12.5%)	8(33.3%)	1(4.2%)	
• Grade III	0	1(5.9%)	10 (58.8%)	5(29.4%)	
Lymphnode status					
• Positive	7 (29.1%)	3 (12.5%)	8(33.3%)	4(16.6%)	0.937
• Negative	2(40%)	1 (20%)	2(40%)	0	
• Not available	7 (33.3%)	1 (4.7%)	9 (42.9%)	2(9.5%)	

and HER2 equivocal which belonged to IDC (NOS) type of grade 1and 2. (Table 2)

In the present study, triple negative cases were more accounting for 38% when compared to other studies. Special variants like medullary carcinoma (8%), secretory carcinoma (2%) and metaplastic carcinoma (2%) which showed triple negativity, were included in our study. Of the 19 Triple Negative Breast Carcinomas (TNBCs), 13 cases were below the age of 50 years which is a risk factor for TNBC [2]. Ten cases showed histological grade 3 with necrosis which could be Basal like breast carcinomas (BLBCs) and belonged to triple negative immunohistochemical subtype. Among 2 cases of ILCs, one case had bilateral presentation at the age of 15 with family history of breast carcinoma and may be of BRCA mutation related breast cancer. TNBC/BLBC phenotype is particularly associated with BRCA1 mutation [9]. These TNBCs further need to be evaluated for basal like expression of basal cytokeratin such as CK5/6 and CK17.

Satti MB et al. [8], Ayadi L et al. [7] and other studies in literature demonstrated high ER, PR positivity with IDC (NOS), ILC and mucinous carcinomas. In the present study also similar observation was made. IDC papillary mucinous type showed ER+ PR-. In the study done by Satti et al, secretory carcinoma was positive for ER and PR but in our study it showed triple negativity with false positive staining of secretions for ER. Although a few cases have been reported that were positive for a hormone receptor, either ERor PR, secretory breast carcinomas usually stain negative for ER, PR and ERBB2 (formerly HER2/neu) (triple-negativebreast carcinoma) [10].

In our study one case of intracystic papillary carcinoma (IPC) was positive for both ER and PR and negative for HER2. In the study done by Wynveen et al, all IPCs (40 of 40; 100%) and associated Invasive carcinoma (IC) were strongly and diffusely ER positive and most (37 of 40; 93%) were also PR positive. All tumors were HER2 negative (score 0/1+) [11].

In the study conducted by Pettinato G. et al.[12] on 62 Invasive micropapillary carcinomas,18 tumors were estrogen receptor–positive (32%); 11 were progesterone receptor–positive (20%); HER2/neu was overexpressed in 53 (95%). In our study we had 2 cases of the same histologic type and both showed ER/PR – (0%) and HER2 + (100%).

In the present study 4 cases of medullary carcinoma and one case of metaplastic carcinoma were triple negative and the findings were in consistent with the study done by Satti MB et al [8] and Ayadi L et al. [7] (Table 3).

Table 2: Immunohistochemical subtypes

Immunohistochemical subtypes	Satti MB et al[6](%)	Onitilo AA et al[12] (%)	Huang J H et al[11] (%)	Present study (%)
ER/PR+, HER2-	53	68.9	66.4	32
ER/PR+, HER2+	11	10.2	30.9	10
ER/PR-, HER2-	24	13.4	13.8	38
ER/PR-, HER2+	12	7.5	45.6	12
ER+/PR-,HER2-	-	-	-	04
ER+/PR+,HER2 equivocal	-	-	-	04

Table 3: Comparison of ER, PR and HER-2, status in varioustypes of carcinoma breast

Histologic subtypes	Satti MB et al[6] (%)			Ayadi L et al[5] (%)			Present study (%)		
	ER	PR	HER2	ER	PR	HER2	ER	PR	HER2
IDC (NOS)	63	63	25	61.1	53.8	16.8	59.5	56.8	21.6
Medullary carcinoma	0	0	0	0	0	0	0	0	0
Invasive Lobular Carcinoma	100	100	2	50	50	16.7	33.3	33.3	33.3
Invasive micropapillary Carcinoma	-	-	-	-	-	-	0	0	100
IntracysticPapillary Carcinoma	-	-	-	-	-	-	100	100	0
IDC with Papillary mucinous type	-	-	-	-	-	-	100	0	0
Metaplastic carcinoma	0	0	0	-	-	-	0	0	0
Secretory carcinoma	100	100	0	-	-	-	0	0	0
Mucoid carcinoma	83	83	0	60	60	0	-	-	-
Apocrine carcinoma	0	0	0	-	-	-	-	-	-
Pleomorphic lobular	100	100	0	-	-	-	-	-	-
Endocrine carcinoma	-	-	-	66.7	33.3	33.3	-	-	-
OncocyticCarcinoma	-	-	-	0	0	100	-	-	-

Table 4: Correlation of ER,PR and HER-2 status in various types of carcinoma breastwith clinicopathological data

Clinicopathological data	Ayadi L et al[5]						Present study					
	ER + %	P value	PR + %	P value	HER2+(%)	p value	ER + %	P value	PR + %	P value	HER2+(%)	p value
Age (years)												
≤ 50	47.5	0.002	53.8	0.76	21.3	0.28	52.8	0.529	50	0.363	22.2	0.791
>50	72		51.4		14.7		42.9		35.7		21.4	
Tumour size (cms)												
≤5	62.7	0.129	53.4	0.72	15.3	0.104	53.7	0.269	48.8	0.400	22.0	0.890
>5	48.6		50		27		33.3		33.3		22.2	
Histologic type												
Infiltrating ductal	61.1	0.31	53.8	0.47	16.8	0.33	59.5	0.072	56.8	0.028	21.6	0.890
Infiltrating lobular	50		45.8		25		33.3		33.3		33.3	
Others							20.0		10.0		20.0	
Lymph node												
Positive	60	0.88	54.7	0.66	36.9	0.000	50	0.884	45.8	0.787	29.2	0.713
Negative	58.9		51.1		4.4		60		60.0		20.0	
Tumour grade												
Grade 1-2	72.2	0.000	61.4	0.000	14.8	0.072	69.7	0.000	66.7	0.000	15.2	0.180
Grade 3	22.5		27.5		27.5		11.8		5.9		35.3	

Table 5: Comparison of Immunohistochemical subtypes with Histologic grading

Immunohistochemical Subtypes(%)	Onitilo AA et al [12] Histologic Grades				Present study Histologic Grades			
	Grade I	Grade II	Grade III	P value	Grade I	Grade II	Grade III	P value
ER/PR+, HER2-	28.9	44.9	21.5	< 0.001	37.5	62.5	0	0.003
ER/PR+, HER2+	6.0	41.4	49.1		20	60	20	
ER/PR-, HER2-	4.0	12.5	76.3		5.3	42.1	52.6	
ER/PR-, HER2+	1.2	20.0	77.7		0	16.7	83.3	

In the present study, ER + tumors were more in the age group of < 50 years. Whereas in the study done by Ayadi et al. [7], ER + tumours were more in the age group > 50 years and Satti MB et al. [8] had similar proportion of ER + cases above and below the age group of 50 years. PR + tumors were more in the age group of ≤ 50 years which was similar to the observation made by Ayadi L et al. [7]. A negative correlation between ER, PR expression and histological grade was noted (p=0.000). No association was found between ER, PR expression and tumor size, histologic type and lymphnode involvement which was similar to the observation made by Ayadi L et al. [7] (Table 4).

In the present study, HER2/neu overexpression was seen in 35.3% of histological grade 3 carcinomas and 15.2% cases of histological grade 1 and 2. Similar observation was made by Ayadi et al. [7] and Huang HJ et al. [13]. There was no correlation between HER2 status and tumor size, histological type, lymph node status and age at diagnosis in the present study and correlated with the study done by Ayadi et al. [7] except that a significant correlation between lymph node status and HER2 overexpression was observed by Ayadi et al (Table 4).

In the present study, we observed a good correlation between tumor grade and immunohistochemical subtypes which was similar to the study done by Onitilo AA et al. [14]. HER2+ and basal like subtypes were associated with poorly differentiated tumors (p=0.003) (Table 5).

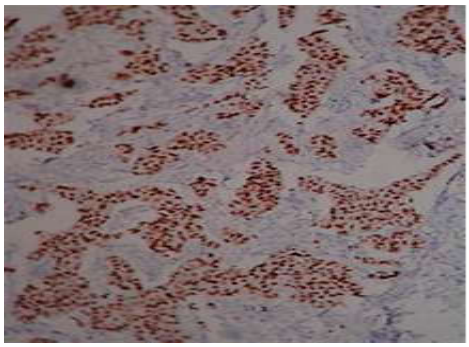

Fig. 1: IDC Grade 1 – ER/PR Positive

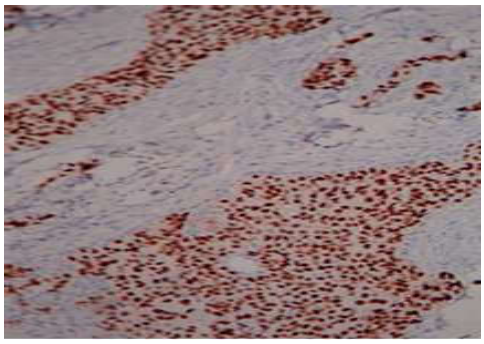

Fig. 2: IDC Grade 2 – ER/PR positive

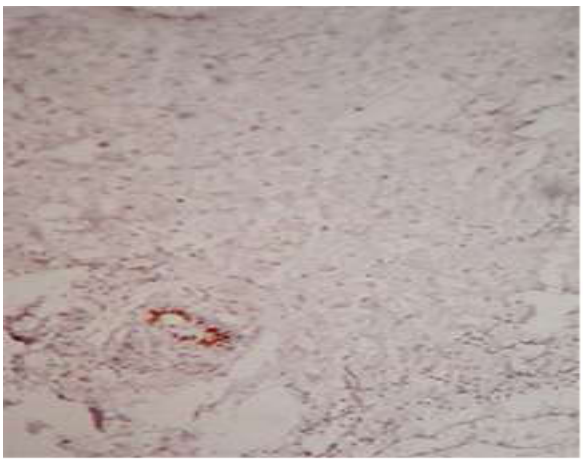

Fig. 3: IDC Grade 3 – Negative for ER/PR(Adjacent Normal Gland Positive for ER/PR)

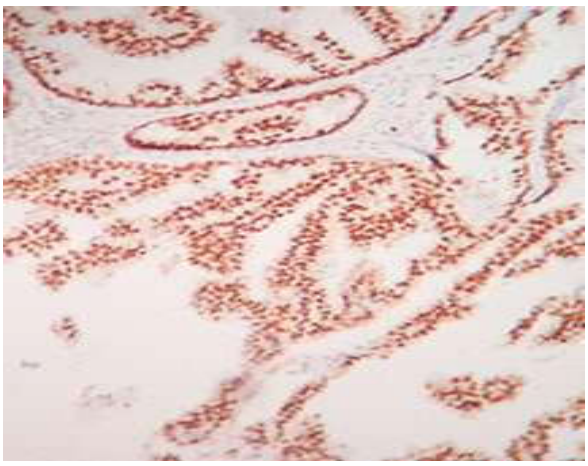

Fig. 5: Intracystic Papillary Carcinoma- ER/PR Positive.

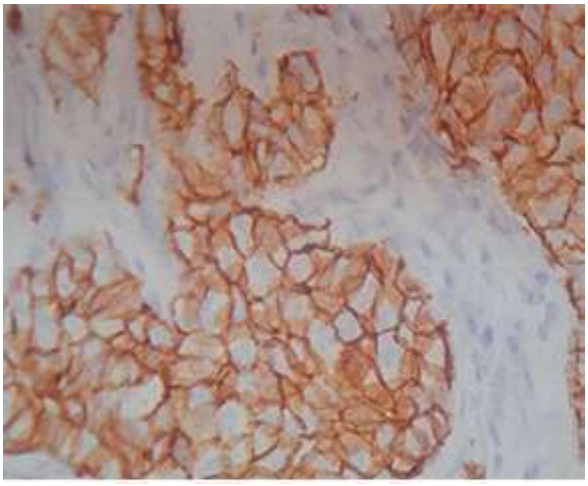

Fig. 4: IDC Grade 3 – Her2/neu Positive

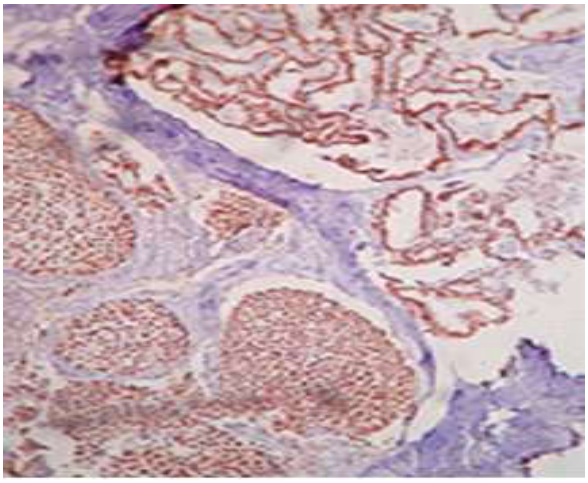

Fig. 6: DCIS Component Positive for ER and PR.

Conclusion

In this study an attempt was made to understand the correlation of ER, PR & HER-2 status with histopathological grading and clinicopathological parameters.

In conclusion, ER, PR and HER-2 status correlates well with histopathological grading. Higher the tumor grade, the more likely that ductal carcinoma will be Her2 + and ER/PR negative or triple negative.

Hence, we support IHC classification as a clinical tool as ER/PR and Her2 testing is widely available at a reasonable cost, is a clinically-used,therapeutically informative classification of breast cancer based on immunophenotype / biologic phenotypes, and is prognostic as well as somewhat predictive. Follow up study of these patients is needed to assess the prognostic significance.

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