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A study of histomorphology and immunohistochemical profile in breast cancer in a tertiary care hospital

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Abstract

The most common cancer among women in the urban Indian population is breast cancer and it is second only to cancer of the cervix in the rural population. Breast cancer is no longer seen as a single disease but rather a multifaceted disease comprised of distinct biological subtypes with diverse natural history, presenting a varied spectrum of clinical, pathologic and molecular features with different prognostic and therapeutic implications. One important aspect of the role of Pathology in the evaluation of breast cancer is biomarker testing, specifically the accurate assessment of the Estrogen receptor (ER), progesterone receptor (PR), and Her2 neu status of a patient's breast cancer. Biomarkers can be prognostic, predictive, or both. Prognostic biomarkers are independent measures of prognosis such that the presence or absence of the biomarker is associated with a patient's overall clinical outcome.

Keywords: Breast carcinoma, ER, PR, Her2 neu, triple negative

Introduction

Breast carcinoma is a malignant proliferation of the epithelial cells lining the ducts and lobules of breast. Breast cancer is the most common malignancy in women, affecting one in 12 in the western world. Advances in imaging techniques and the increased use of aspiration needle biopsy have greatly assisted preoperative evaluation of breast lesions; however, in a large proportion of cases, differentiation between benign and malignant lesions still rests on histologic examination. Currently, routine clinical management of breast cancer incorporates specific molecular markers; namely ER (Estrogen receptor), PR (progesterone receptor), Her 2 neu (human epidermal growth factor receptor 2 gene) that have been proven to provide therapeutic, predictive and prognostic value.

Aims and Objective

1. To analyse histomorphology and lymph node status in breast carcinomas.
2. To study Estrogen receptor (ER), progesterone receptor (PR) and human epidermal growth factor receptor 2 (Her2 neu) in all these breast carcinomas

Materials and methods

All the lumpectomy, simple mastectomy and modified radical mastectomy specimens of carcinoma breast submitted to the Department of Pathology in a tertiary care hospital in 1 year were enrolled. A total of 50 cases were included in this study. H&E sections from the tumour were analysed for histomorphology and corresponding tumour section was used for immunohistochemistry analysis of ER, PR and Her2neu.

Observation and results

The present study comprised of 50 cases of breast carcinoma over a period of 24 months. The age of the patients range from 32 to 77 years with a mean age of 51.10 years (SD 10.869).

Majority of patients were in age group of 40-49 years. Modified radical mastectomies (96%) comprised the maximum load of specimens received followed by small biopsies (4%). Most of the lesions were in the Right breast (64%). The most common location of tumour was in upper outer quadrant with 22 cases (44%) followed by upper inner quadrant with 11

cases (22%). In our study, the size of the tumour ranged from 1 to 9 cm with majority of the tumours (50%) in the size range of 2 to 5 cm. Most common histologic type was Infiltrating Ductal Carcinoma (Not otherwise Specified) with 66% cases (Table 1) (Figure1). Majority of the cases were of Grade II with 39 (78%) cases (Table 2). Lymph node positivity was seen in 24 cases (48%) and in 4 cases (8%) more than 10 positive lymph nodes were found.

ER, PR, Her 2neu status (Figure 2-4)

The immunohistochemical analysis revealed 54% (27 cases) positivity for ER with Allred score of 3-8. Majority (62.96%) of the ER positive cases had the Allred score of 7 or 8. PR positive cases were 23 (46%) with Allred score of 7 or 8 in most of the positive cases. Her2 neu was positive in 11 (22%) cases. Equivocal score was seen in 3 (6%) cases.

Lymph node status and Allred score for ER: In 24 cases (48%) lymph nodes were involved in which 12 cases (50%) were ER negative (Allred Score: 0,2). Lymph nodes were not involved in 24 cases (50%) in which 13 cases (54%) had ER positivity (Allred score: 3-8) (Table 3). Significant association was noted with ER score and lymph node involvement (p value < 0.05).

Lymph node status and Allred score for PR: In 24 cases (48%) lymph node status was positive with 13 cases (54.2%) being PR negative. In lymph node negative cases, 10 cases (41.6%) were PR positive (Allred score 3-8) (Table 4).

Lymph node status and Her2 neu score: In 36 (72%) cases Her2 neu score was negative. In HER2 neu negative cases, lymph nodes were involved in 17 patients (47.22%). Equivocal status was seen in 3 cases in which 1 case was positive for lymph nodes. In 11 patients HER2 neu score was 3+ out of which 6 cases (54.5%) had lymph node involvement (Table 5).

Allred score for Pr with her2/NEU score

In 11 cases (22%) HER2/neu was positive in which 10 cases (90.9%) were negative for PR with Allred score of 0 or 2. HER2/neu score inversely correlated with the Allred Score for PR (Table 6). Pearson Chi Square test showed significant association between HER2 neu score and Allred score for PR.

Triple negative status and histologic grade: The study had 13 triple negative cases, of which 12 cases (92.3%) were grade II or grade III, thus emphasising that Triple negative status is associated with higher grade (Table 7).

Pearsons Chi Square test showed significant correlation among triple negative status and tumour grade (p value < 0.05).

Discussion

Age: The age range of present study with mean age of 51.10 years was comparable with various other studies (Table 8).

Laterality: The present study had majority of tumours in the right breast while the studies by Sofi *et al.* and Nisa *et al.* have left predominance. (Table 9)

Tumour Size: In the present study the size of tumour ranged from 1 to 9 cm. The mean tumor size was 3.52 cm and is comparable to the studies by Mudduwa *et al.* [1] and Sofi *et al.* [2] (Table 10). Also it is noted in the present study that 72% of cases showed more than 2 cm tumor size which is similar to the results observed by Bhagat Vasudha *et al.* [3] (91.38%), Azizun *et al.* [4] (88%), Moses *et al.* [5] (91.6%) and Mona *et al.* [6] in their study. While study from western country, Adedayo *et al.* [7] showed 71.4% cases with ≤ 2 cm size, this could be due to early cancer detection programs. In India owing to the lack of awareness of this disease and absence of a breast cancer screening program, the majority of breast cancers are diagnosed at a relatively advanced stage. In our study 14.3% of tumours which were less than 2 cm in size showed HER2/neu positive staining, 20% cases in the size range of 2-5 cm were HER2/neu positive and 36.4% cases in which tumour size was more than 5 cm were HER2/neu positive. Significant association between increasing tumor size and HER-2/neu expression was seen in our study. Tumors larger than 2 cm size had higher rates of Her-2/neu expression than those of tumors less than 2 cm size. Similar result was observed by Bhagat *et al.* [3] in their study.

Histologic type: The most common histologic type in the present study was IDC-NOS which is comparable to the findings of other studies (Table11).

Tumour grade: The most common tumour grade in the present study was grade II with 78% cases followed by grade I with 16% cases. This was similar to findings by Sofi *et al.* [2] and Ayadi *et al.* (Table 12).

Lymph node status: In 24 (48%) cases lymph nodes were positive for metastasis. In 2 cases lymph nodes were not retrieved. Lymph nodes were not involved in 24 (48%) cases. In the studies by various authors like Zubair Ahmed *et al.* [8], Mudduwa *et al.* [11], Seho Park *et al.* [9], Lobna Ayadi *et al.* [10] and Lokuhetty [11] documented lymph nodes positive for metastasis as 74.77%, 57.7%, and 27.8%, 65% and 41% respectively.

ER/PR and HER2/neu status: Comparison of ER/PR and HER2/neu status in various studies is shown in the table 13. Out of total 50 cases, 22 cases (44%) showed positive staining for both ER and PR and 21 cases (42%) were negative for both ER and PR. Our findings were in concordance with study of R. Kumawat [12], who demonstrated 33% cases showed positive staining for both ER and PR while 45% cases showed negative staining for both estrogen and progesterone receptors. The negative ER PR status in IDC-NOS was comparable to study by Kumawat *et al.* [12].

HER2/neu score and Histologic Type: We compared our results with previously published international data. In the Sloan-Katterring study HER-2 positivity was limited to infiltrating breast carcinoma of the ductal and lobular morphology. None of the special type carcinomas like mucinous, medullary or papillary showed HER-2 positivity. These findings were similar to our study.

Histologic Grade with ER/PR and HER2/neu expression:

The comparison of molecular marker expression in different studies are given in the table 14. Our findings were in concordance with Bird P *et al.* [13] who demonstrated that increasing tumour grade is associated with decreased PR expression

Triple Negative Breast Carcinoma (TNBC): The present study had 26% of triple negative breast carcinomas (TNBC). A major proportion (30.7%) of TNBC had tumour size >5cm as compared to 18.91% in other cases. The finding that TNBC present with larger size compared to other group is consistent with findings by Bauer *et al.* [14], Li *et al.* [15], Somali *et al.* [16]

Also the rate of node positivity was found in TNBC was high (53.84%) compared to node positivity in other group of carcinomas (45.94%). This is consistent with findings of Bauer *et al.*, Li *et al.* The majority of TNBC were of grade III, the finding being consistent with Carey *et al.* [17], Bauer *et al.* [14], Somali *et al.* [16].

There are conflicting reports in literature regarding lymph node status in TNBC. While some publication report a higher rate of node positivity (Bauer *et al.* 2008; Li *et al.* 2013) [14, 15] Some report node negativity more common in TNBC (Foulkes *et al.*, 2003; Cheang *et al.*, 2008; Somali *et al.* 2013) [18, 19, 16]. In our study a high rate of node positivity was found in TNBC compared to other group (53.84% vs 45.94%). Comparing the histological subtypes, IDC NOS comprised maximum number of cases in both the study groups (61.53% vs 67.56%). Similar results were observed in other studies (Carey *et al.* 2006; Somali *et al.* 2013) [17, 16].

Conclusion

The study of ER, PR and HER2 neu expression in breast carcinomas by IHC is a very important diagnostic tool in deciding the treatment strategy and the prognosis. The study throws a light on lack of adequate screening programs and awareness regarding breast carcinoma in India compared to western countries. The study establishes a significant correlation between lack of estrogen receptors and lymph node involvement and also that expression of HER2 neu

receptors are inversely related to the expression of progesterone receptors. Triple negative status of tumours significantly associated with higher tumour grade and tumour size.

Table 1: Distribution of breast carcinomas according to histologic type

Histological type	Frequency	Percentage
IDC-NOS	33	66.0
IDC with DCIS	5	10.0
Multicentric IDC	1	2.0
IDC with medullary component	2	4.0
Invasive papillary carcinoma	1	2.0
Invasive lobular carcinoma	3	6.0
Invasive medullary carcinoma	1	2.0
IDC with paget's disease	1	2.0
Mucinous carcinoma	2	4.0
DCIS	1	2.0
Total	50	100.0

Table 2: Distribution of breast carcinomas according to histologic grade

Histological grade	Frequency	Percentage
Grade 1	4	8
Grade 2	39	78
Grade 3	7	14
Total	50	100

Table 3: Lymph node status and Allred score for ER

Allred score for ER		Lymph Node status			Total
		Not retrieved	Negative	Positive	
0,2	Frequency	0	11	12	23
	%	0.0	45.8	50.0%	46.0%
3	Frequency	1	0	0	1
	%	50.0	0.0	0.0%	2.0%
4-6	Frequency	0	6	3	9
	%	0.0	25.0	12.5%	18.0%
7-8	Frequency	1	7	9	17
	%	50.0	29.2	37.5%	34.0%
Total	Count	2	24	24	50
	%	100.0%	100.0%	100.0%	100.0%

Table 4: Lymph node status and Allred score for PR

Allred score for PR		Lymph Node status			Total
		Not retrieved	Negative	Positive	
0,2	No. of Cases	0	14	13	27
	%	0.0	58.3	54.2	54.0
3	No. of Cases	0	1	1	2
	%	0.0	4.2	4.2	4.0
4-6	No. of Cases	1	5	2	8
	%	50.0	20.8	8.3	16.0
7-8	No. of Cases	1	4	8	13
	%	50.0	16.7	33.3	26.0
Total	No. of Cases	2	24	24	50
	%	100.0	100.0	100.0	100.0

Table 5: Lymph node status and Her2/neu score

Lymph node status		Her2 neu				Total
		Negative	Negative 1+	Equivocal 2+	Positive 3+	
Not retrieved	No. of Cases	2	0	0	0	2
	%	5.9	0.0	0.0	0.0	4.0%
Negative	No. of Cases	17	0	2	5	24
	%	50.0	0.0	66.7	45.5	48.0%

Positive	No. of Cases	15	2	1	6	24
	%	44.1	100.0	33.3	54.5	48.0%
Total	No. of Cases	34	2	3	11	50
	%	100.0	100.0	100.0	100.0	34.0%

Table 6: Allred Score for PR with HER2/neu Score

Allred Score for PR		Her2 neu				Total
		Negative	Negative 1+	Equivocal 2+	Positive 3+	
0,2	No. of Cases	15	1	1	10	27
	%	44.1	50.0	33.3	90.9	54.0%
3	No. of Cases	1	1	0	0	2
	%	2.9	50.0	0.0	0.0	4.0
4-6	No. of Cases	7	0	1	0	8
	%	20.6	0.0	33.3	0.0	16.0%
7-8	No. of Cases	11	0	1	1	13
	%	32.4	0.0	33.3	9.1	26.0%
Total	No. of Cases	34	2	3	11	50
	%	100.0	100.0	100.0	100.0	100.0

Table 7: Triple negative status and histologic grade

ER, PR & Her2/neu status		Histologic grade			Total
		Grade I	Grade II	Grade III	
Triple Negative	No. of Cases	1	8	4	13
	%	25	20.5	57.14	26.0
Others	No. of Cases	3	31	3	37
	%	75	79.5	42.8	74.0
Total	No. of Cases	4	39	7	50
	%	100.0	100.0	100.0	100.0

Table 8: Age range and Mean age of carcinoma breast in various studies

Study	No. of cases	Age range (years)	Mean age (years)
Present Study	50	32-77	51.10
Ahmed <i>et al.</i> [8]	157	16-80	43.75
Cadman <i>et al.</i> [20]	103	31-83	59
Mudduwa <i>et al.</i> [1]	151	31-85	52.5
Konofaos <i>et al.</i> [21]	119	36-86	50
Faheem <i>et al.</i> [22]	1226	27-84	48.04
Mohsin <i>et al.</i> [23]	1042	25-80	45.6

Table 9: Tumour laterality in various studies

	Left	Right	Bilateral
Present study	36%	64%	-
Sofi <i>et al.</i> [2]	50.8%	48.5%	0.7%
Nisa <i>et al.</i> [24]	57%	43%	-

Table 10: Tumour Size in various studies

Study	Mean tumour size (in Centimeter)
Present study	3.52
Mudduwa <i>et al.</i> [1]	3.52
Sofi <i>et al.</i> [2]	3.56
Cadman <i>et al.</i> [20]	2.1

Table 14: Histologic Grade with ER/PR and HER2/neu expression

Study	Grade I		Grade II		Grade III	
	Present Study	K Rathod <i>et al.</i> [28]	Present Study	K Rathod <i>et al.</i> [28]	Present Study	K Rathod <i>et al.</i> [28]
ER	66.6	68.7	59	27.02	0	28.5
PR	66.6	62.5	46.2	27.02	20.0	28.5
Her 2 neu	0	6.25	38.5	18.9	0	71.42

Table 11: Comparison of various histologic types in other studies

	IDC	ILC	IMC	IPC
Present Study	84%	6%	2%	2%
Zoppi <i>et al.</i> [25]	95%	2%	-	1%
Malaviya <i>et al.</i> [26]	97.5%	0.5%	-	1%
Stalhammer <i>et al.</i> [10]	80%	18%	-	-

Table 12: Histologic grade in various studies

Study	Grade I	Grade II	Grade III
Present Study	6%	78%	10%
Ahmed <i>et al.</i> [8]	25.3%	55.2%	19.5%
Mudduwa <i>et al.</i> [1]	14.6%	36.4%	49%
Sofi <i>et al.</i> [2]	7.6%	52.1%	40.3%
Ayadi <i>et al.</i> [10]	10.9%	63.2%	25.8%

Table 13: Comparison of ER, PR and HER2/neu status in various studies

S. No	Study	ER + (%)	PR + (%)	Her2/neu + (%)
1	Present study	54.0	46.0	22.0
2	Faheem <i>et al.</i> [22]	62.2	60.9	38.9
3	Mostafa <i>et al.</i> [27]	69.0	72.3	28.4
4	R.Kumawat <i>et al.</i> [12]	52.0	34.0	-
5	K Rathod <i>et al.</i> [28]	32.7	25.3	24.7

Table 15: Triple Negative Breast Carcinoma (TNBC) in various studies

Study	TNBC cases (%)
Present Study	26
Syeda Zubeda <i>et al.</i> [29]	46
Ghosh <i>et al.</i> [30]	29.80
Rakha <i>et al.</i> [31]	16.30
Dunwald <i>et al.</i> [32]	25
Bauer <i>et al.</i> [14]	12.5

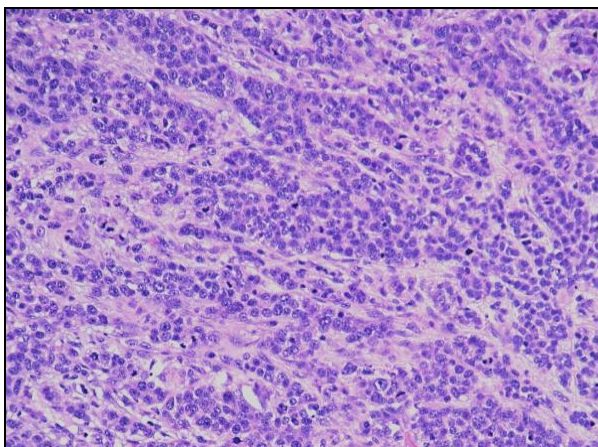


Fig 1: Infiltrating ductal carcinoma (grade III) [H&E, 100X]

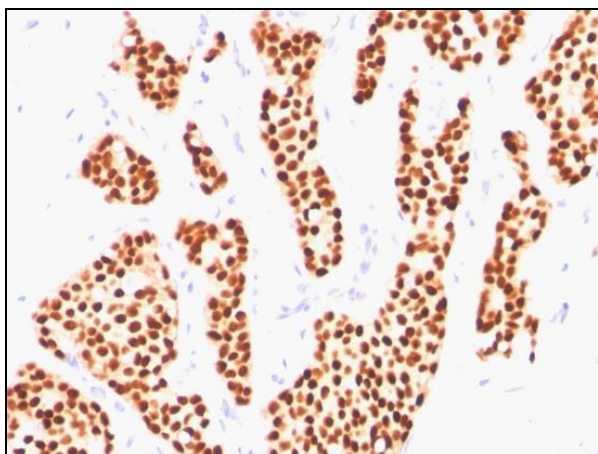


Fig 2: Strong nuclear staining for ER (Allred score: 8) [IHC-ER, 400X]

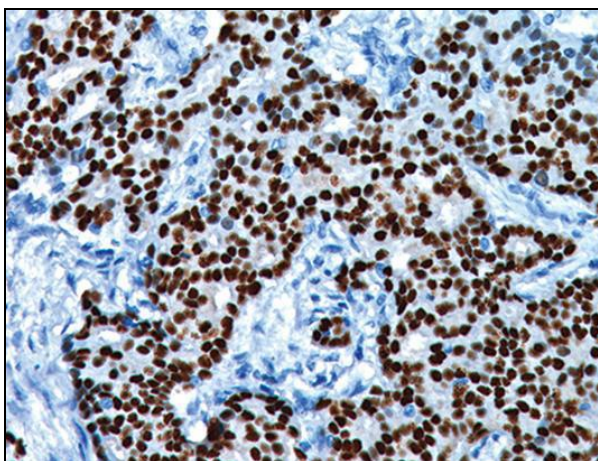


Fig 3: Strong nuclear staining for PR in infiltrating ductal carcinoma (Allred score: 8), [IHC- PR, 400X]

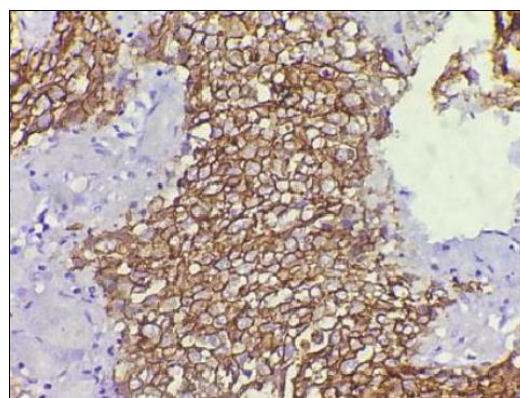


Fig 4: Strong complete membrane staining for HER2/neu (3+), [IHC-HER2/neu, 400X]

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