

## Original Research Article

## Study of Association Between Human Epidermal Growth Factor Receptor2/neu Expression With Modified Bloom Richardsons Grading in Breast Cancer at A Tertiary Care Hospital Puducherry

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**Abstract**

*Context:* Breast cancer is the most common malignancy in females. Advancement in cancer management had lead to early detection and treatment of disease. Recently Immunohistochemistry plays an vital role in the classification of breast cancer. Estrogen Receptor/Progesterone Receptor and Human epidermal growth factor receptor/neu (Her2/neu) status provides prognostic and therapeutic information. *Aims:* This study is done to find the association between Her2/neu by immunohistochemistry and various clinicopathological factors in breast cancer. *Study design:* The present study is a cross sectional study. *Material and Methods:* Forty patients who were diagnosed to have carcinoma breast cancer patient underwent Modified radical mastectomy were included in the study. The surgical specimen were then evaluated histopathologically using modified Bloom Richardsons grading and immunohistochemically for Her2/neu markers. *Results:* In the present study most of the cases were aged between 50-60 years. The mean age of presentation was 52.4. The most common histological type were infiltrating ductal carcinoma no special type, constituting 35 (87.5%). Grade II tumours were seen in 57.5%. Her2 was positive in 16 cases (40%), and it was negative in 26 (60%). Among the positive cases 14 cases were of Grade III. Lymphovascular involvement was seen in 15 cases. *Conclusion:* This study emphasize the need of Her2/neu in routine histopathological report in breast cancers. Her2 positivity increased as the tumour Grade increases and also had increased lymphnode involvement, lymphovascular invasion with poor prognosis.

**Keywords:** Breast cancer; Human Epidermal Growth Factor Receptor2/neu; Tumour grade.

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**Introduction**

Breast cancer is the most common malignancy in females, every year more than 1 million women

are diagnosed with breast cancer.<sup>1</sup> Invasive ductal carcinoma of no special type (NST) is the most common histological type accounts for 60-80% of all cases of breast carcinoma.<sup>2</sup>

In breast carcinoma, several features have prognostic significance including histologic subtype, in situ carcinoma component, tumour grade, lymph node states, resected margin status, skin involvement, expression of estrogen receptor (ER)/progesterone receptor (PR) status, human epidermal growth factor receptor2/neu status, growth factors and its receptors, proliferative activity, oncogenes, and tumour suppressor genes<sup>3</sup>.

Her2 gene encodes a 185 kDa transmembrane phospho-glycoprotein with tyrosine kinase activity and is a member of the human epidermal growth factor receptor gene family. Her2 neu amplification and over expression of gene protein is seen in 10-34% of invasive breast cancer which has been associated with poor prognosis. It also serves as a very useful parameter to predict response to anti Her2 neu targeted therapy with Herceptin which helps in the patients morbidity and mortality.

## Materials and Methods

The present study is carried out in Department of Pathology, Sri Manakula Vinayagar Medical College and Hospital, Puducherry for the period of one and half years both retrospectively as well as prospectively from the period of November 2017 to June 2019 has been included after getting clearance from the institutional ethics committee.

The specimen were fixed in 10% formalin for 24 hours and studied grossly. After paraffin embedding 4  $\mu$  sections was obtained and stained by Haematoxylin & Eosin stain. The lesion are classified and graded as per the Modified Bloom Richardsons Grading system [tubule formation, nuclear pleomorphism and mitosis]. Immunohistochemistry analysis of Her2/Neu scoring was also done for the same.

IHC is done using polymer kit. Her2/Neu was scored using ASCO guidelines in which Score 0 (Negative): No staining is observed or membrane staining is observed in <10% of the tumour cells, Score 1+ (Negative): A faint/barely perceptible membrane staining is detected in more than 10% of the tumour 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 tumour r cells, Score 3+ (Strongly Positive): A strong complete membrane staining is observed in more than 30% (formerly 10%) of the tumour cells.

## Statistical analysis

Data analysis was done using SPSS version 24.2. Description of the study variables will be carried out using frequency and percentage. Association between histopathology and immunohistochemistry of the breast lesion is done by Chi-square test  $p < 0.05$  will be considered statistically significant.

## Results

This study included total of 40 histopathologically proven cases of breast carcinoma. In the present study most of the cases were between 50–60 years comprising about 32.5%. The mean age of presentation was 52.4. Most of the cases were postmenopausal about 27 (67.5%). Family history was present in 4 cases (10%). Past history of exogenous hormone intake was present in 2 (5%) of cases. Majority of cases presented clinically with breast lump 27 (67.5%). Left breast involvement is seen in 26 cases (65%). Most of the tumour was present in inner lower quadrant comprising 40%. Among 40 cases FNAC was done in 21 cases (52.5%) and all were positive for malignancy. Histological correlation is seen in all 21 cases (Table 1).

**Table 1:** Age distribution

Age in years	Number of patients	%
30-40	5	12.5
40-50	11	27.5
50-60	13	32.5
60-70	9	22.5
70-80	2	5.0
<b>Total</b>	<b>40</b>	<b>100.0</b>

Most of the cases were of size 2–5 cm. The most common histological type was infiltrating ductal carcinoma no special type, constituting 35 (87.5%).

Most of the cases were of Grade II constituting about 57.5%, followed by Grade III comprising 42.5% none of the case showed Grade I tumour. In the present

study 27 (67.5%) tumours had desmoplasia, 24 (60%) had necrosis, 21 (52.5%) had LVI, 15 (37.5%) had calcification. Necrosis, desmoplasia, LVI were significantly more in high grade tumors.

The morphological features like desmoplasia,

necrosis, lymph node involvement and lymphovascular involvement were seen to correlate with MBR grading which was found to be Statistically significant with  $p$ -value  $< 0.05$ . Others features were not Statistical significant (Table 2).

**Table 2:** Morphological pattern correlation with MBR grading

Morphological pattern	Modified Bloom Richardsons Grading			<i>p</i> -value
	I	II	III	
<b>Tumor Size (cm)</b>				
<2	0	3 (7.5%)	2 (5%)	0.864
2 - 5	0	13 (32.5%)	11 (27.5%)	
>5	0	7 (17.5%)	4 (10%)	
<b>DSM</b>				
DSM involved	0	3 (7.5%)	6 (15%)	0.096
DSM not involved	0	20 (50%)	11 (27.5%)	
<b>Histologic type</b>				
IDC NST	0	21 (52.5%)	14 (35%)	0.229
IDC+Medullar	0	0	1 (2.5%)	
Medullary	0	0	2 (5%)	
IDC+Neuro	0	1 (2.5%)	0	
IDC+Mucinous	0	1 (2.5%)	0	0.048
Desmoplasia		12 (30%)	14 (35%)	
		11 (27.5%)	3 (7.5%)	
Necrosis	0	7 (17.5%)	17 (42.5%)	<0.05
		16 (40%)	0	
<b>Nodal involvement</b>				
Present	0	7 (17.5%)	17 (42.5%)	<0.05
Absent	0	16 (40%)	0	
<b>Lymphovascular Invasion</b>				
Present	0	7 (17.5%)	14 (35%)	<0.05
Absent	0	16 (40%)	3 (7.5%)	
<b>Adjacent changes</b>				
Fibrocystic change	0	16 (40%)	9 (22.5%)	0.489
Atypical ductal hyperplasia	0	5 (12.5%)	4 (10%)	
Lobulitis.	0	29 (5%)	3 (7.5%)	
Monckeberg's Medial	0	0	1 (2.5%)	
Calcification				

MBR-Modified Bloom Richardsons, DSM-Deep surgical margin.

Her2 was positive in 16 cases (40%), and it was negative in 26 (60%). Among the positive cases 14 cases were of Grade III i.e. 37.5% showed intense complete membrane staining and 1 cases (were

in Grade II with moderate complete membrane staining. Lymphnode involvement was seen in 24 cases among which 15 were Her2+ (Table 3).

**Table 3:** Correlation of HER2 neu to the following clinicopathological features

Parameter	HER2neu		<i>p</i> -value
	Positive	Negative	
<b>Menstrual Status</b>			
Pre-menopause	1 (2.5%)	4 (10%)	0.724
Perimenopause	6 (15%)	5 (12.5%)	

Parameter	HER2neu		p-value
	Positive	Negative	
Postmenopause	9 (22.5%)	15 (37.5%)	
<b>Laterality</b>			
Right	4 (10%)	10 (25%)	0.279
Left	12 (30%)	14 (35%)	
<b>Size (cm)</b>			
<2	2 (5%)	3 (7.5%)	0.957
2-5	10 (25%)	14 (35%)	
>5	4 (10%)	7 (17.5%)	
<b>Hystology Type</b>			
IDC NST	14 (35%)	21 (52.5%)	0.287
IDC+Medullar	0	1 (2.5%)	
Medullary	0	0	
IDC+Neuro	1	1 (2.5%)	
IDC+Mucinous	1 (2.5%)	1 (2.5%)	
<b>Nodal Involvement</b>			
Positive	15 (37.5%)	9 (22.5%)	<0.05
Negative	1 (2.5%)	15 (37.5%)	

HER2- Human Epidermal rowth factor Receptor2/ neu, IDC NST-Infiltrating Ductal Carcinoma no special type

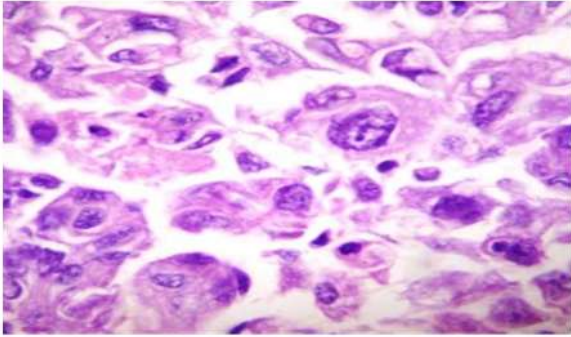
Her2 positivity was seen in 17.5% of premenopausal women and 80% of postmenopausal women. Most of the Her2 positive tumour were in T3 stage constituting 25%. Most common histological type is IDC (NST) with 35%. Her2 positivity with tubule formation and mitotic grade was seen most common in Score 3 it was statistically

significance with a *p*-value <0.05 except for nuclear pleomorphism. Her2 positivity is maximum in Grade III tumour with 14 cases amounting 35%. Lymphnode involvement is seen in 37.5% of Her2 cases. Both Grade and Lymphnode involvement in correlation with Her2 was statistically significance (Table 4).

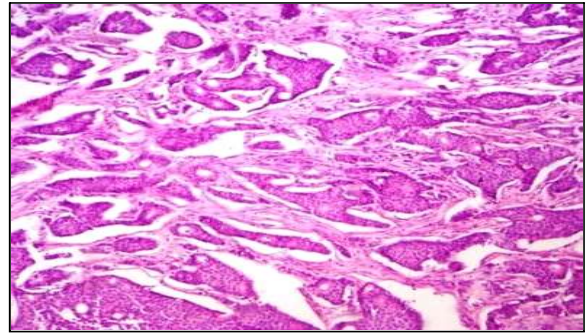
**Table 4:** Correlation of HER to the following MBR histological features

Histological features	Her2		p-value
	Positive	Negative	
<b>Tubule formation</b>			
Score 1	0	0	<0.05
Score 2	1 (2.5%)	20 (50%)	
Score 3	15 (37.5%)	4 (10%)	
<b>Nuclear pleomorphism</b>			
Score 1	0	0	0.146
Score 2	11 (27.5%)	21 (52.5%)	
Score 3	5 (12.5%)	3 (7.5%)	
<b>Mitosis</b>			
Score 1	0	0	<0.05
Score 2	1 (2.5%)	19 (47.5%)	
Score 3	15 (37.5%)	5 (12.5%)	
<b>Grade</b>			
II	2 (5%)	21 (52.5%)	<0.05
III	14 (35%)	3 (7.5%)	

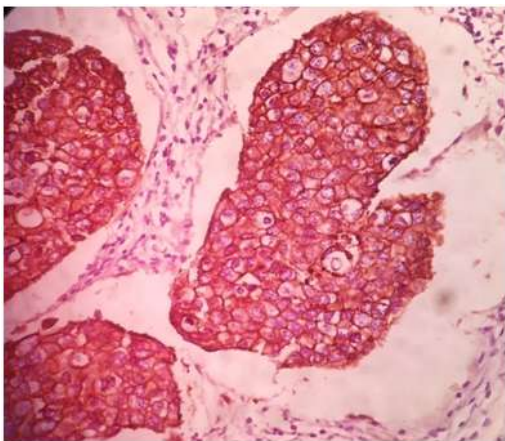
MBR-Modified BloomRichardsons, Her2- Human Epidermal rowth factor Receptor2/ neu



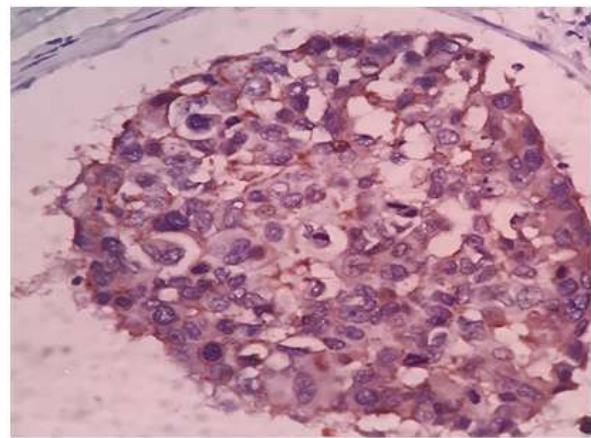
**Fig. 1:** Grade III -IDC(NST) (x400 H & E).



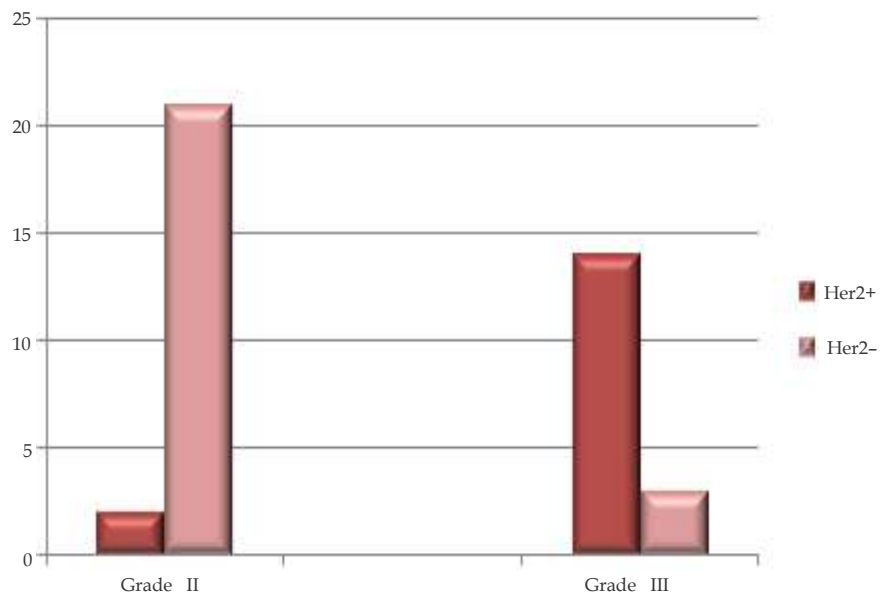
**Fig. 2:** Grade II -IDC(NST) (x100 H & E).



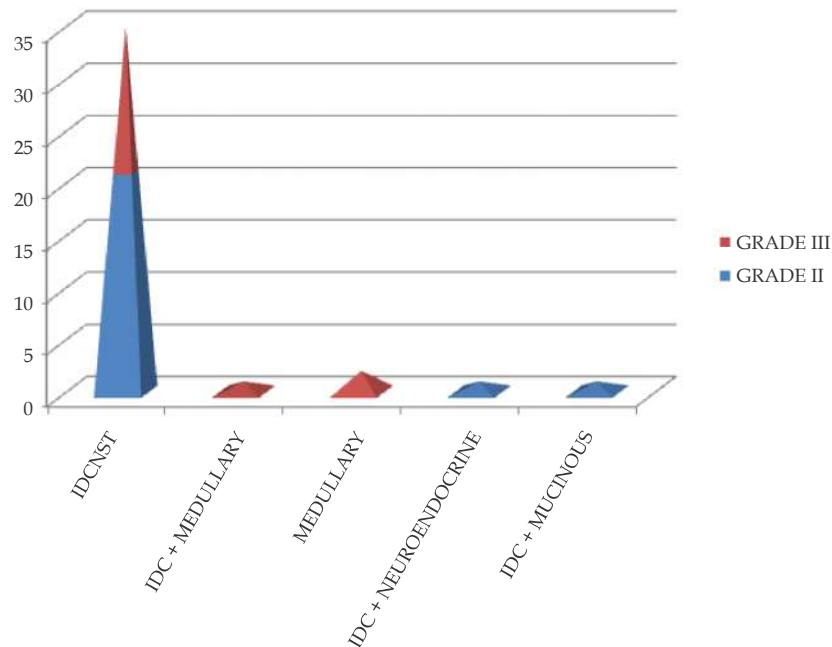
**Fig. 3:** Her2 strong positive, Score 3A strong complete membrane staining is observed in more than 30% of the tumour cells.



**Fig. 4:** Her2 Score 2, A weak-to-moderate complete membrane staining is observed in more than 10% of the tumour r cells.



**Graph 1:** Association of Her2 with Grade.



**Graph 2:** Histological subtype and grading.

## Discussion

Breast cancer is the most common cancer among women in India. The use of IHC has got its pivotal value for the complete and comprehensive histopathology report in breast cancer. To know the prognostic and predictive markers of breast carcinoma many research have been done. Among many prognostic markers ER, PR and Her2/neu are the most important and helps the clinicians to decide further management. This study has been done to evaluate the association of modified Bloom Richardson's histological grading and Her2 neu receptor status of breast cancer which can aid the patient for further targeted therapy.

The age of patients ranged from 31 to 75 years, with a mean age group of 52.4 years. Maximum numbers of cases were seen in the age group of 50–60 years (32.5%) years. No cases were seen in the age group of 21–30 years. This finding was similar to the study done by Thiagarajan M et al.,<sup>4</sup> Rao, et al.<sup>5</sup> and Meena et al.<sup>6</sup> In this present study most common quadrant involved is lower inner (40%) which is contradictory to other studies in which it was upper outer quadrant.

In our study most of the tumour were of size ranging from 2–5 cm, this finding was similar to the study done by Thiagarajan M et al., Badwe et al.,<sup>7</sup> Azizun-Nisa et al.<sup>8</sup> states that Her2 positivity increases with tumour size, in the present study no significant changes demonstrated with Her2/neu

to size of the tumour. Similarly G. R. Molina Barrios et al.<sup>9</sup> states that there is no correlation of Her2/neu with tumour size.

The most common histological type was infiltrating ductal carcinoma no special type, constituting 35 (87.5%). It is followed by 2 cases (5%) medullary carcinoma, 1 case (2.5%) each of invasive ductal with medullary features, invasive ductal carcinoma with neuroendocrine features and invasive ductal carcinoma with mucinous differentiation. Similar findings were observed by Thiagarajan M et al. (87.5%) and Mushood et al. (90.6%).<sup>10</sup> Other types seems to have varied incidence in different studies.

Most of the cases were of Grade II constituting about 57.5% followed by Grade III comprising 42.5% none of the case showed Grade I tumour which was similar to the study done by Thiagarajan M et al. and OnitiloAA et al.<sup>11</sup> This study states that when as the grade of the tumour increases Her2 expression significantly increased and well differentiated tumours shows decreased expression of Her2. Similar observations was seen in studies done by Ayadi L et al. and Thiagarajan M et al.

## Conclusion

From the present study it was concluded that Infiltrating Ductal carcinoma (NST) was the most common histological sub type of breast cancers. As

the tumour grade increases Her2/neu expression also increases. Her2 is also seen increased in tumour with lymphnode and lymphovascular involvement. Prognostic accuracy improves with incorporation of IHC into the histopathology report along with the traditional TNM staging and histological grading.

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