Original Research Paper

Histopathology

CORRELATION OF EXPRESSION OF CK5/6 AND HER 2 WITH HISTOLOGICAL GRADES OF BREAST CARCINOMA AND NOTTINGHAM PROGNOSTIC INDEX SCORES

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ABSTRACT

BACKGROUND: Breast Carcinoma is very rampant in the world and has been associated with most deaths in the females in India. Targetted therapies help in increasing the survival rates of the affected females since the age of presentation is highly variable and this can be achieved by categorizing the lesions morphologically and through various immunohistochemial markers.

AIM: The aim of this study is to study the expression of CK 5/6 and HER 2 in varying grades of Breast Carcinoma and correlate it with the Nottingham Prognostic Index.

METHODS: This study was conducted in Chengalpattu Medical College and Hospital as a prospective and retrospective record based study among patients with Breast malignancies diagnosed as Invasive ductal carcinoma. Patients who underwent surgery after chemotherapy and radiotherapy and those with mesenchymal/Metastatic tumors were excluded. A total of 70 breast cancer cases for which biopsy blocks were retrieved from the Department of Pathology. Sections were studied and graded based on TNM staging criteria. Data analysis was done.

RESULTS: The correlation of NPI with HER2 expression was found to be statistically significant. The marker positivity of CK 5/6 and HER 2 were analysed with Grade and NPI as dependent variables. Though not statistically significant, the CK 5/6 strong positive tumors along with HER2 positivity was quite higher in Grade I tumors(10 cases) compared to Grade II tumors(3 cases).

CONCLUSION: Though studies available indicate expression of CK5/6 and HER2 indicate aggressive lesions , no statistical correlation was found between Higher grade and the marker expression in our study. However this study shows the strong positivity of CK5/6 in Good prognostic Group which also needs to be followed up and treated aggressively even though their grade and NPI score is low. Hence this underlines the fact that Basal 2- HER2 subtypes should be given more importance and Immunohistochemical analysis is a must even in cases of lower histopathological grades and NPI scores.

KEYWORDS : Breast Carcinoma,CK 5/6, HER2

INTRODUCTION

Breast cancer is the most commonly occuring cancer in females with more than 1.7 million cases around the world[1]. It represents 12% of new cancers arising every year and 25% of all cancers in women. The most common age group of presentation worldwide is 50-60 years. Indian women with breast cancers are found to be younger by a decade and present more aggressively. Studies suggest that the peak incidence in Indian women is 40-50 years.

Initially due to the stigma, a lot of cases went unnoticed but now due to the widespread health education and screening programmes the detection rate and those undergoing treatment has substantially increased[2]. The triple assessment of palpation, radiological evaluation and pathological examination has increased the early detection of breast malignancies[3].

Morphological examination and Immunohistochemistry plays a vital role in diagnosis and prognostication of breast carcinomas. Lymph node assessment from gross examination and microscopic features helps in assessing the stage of the disease. Molecular subtypes of breast malignancies are classified using ER, PR, HER2 status and basal markers routinely, in trucut samples as well as mastectomy specimens and accordingly protocols of treatment have been devised to suit the patient.

Newer modalities have been achieved in the form of breast conservative surgeries, neoadjuvant chemotherapy and the hormonal therapies have evolved for the betterment and thereby improving the longevity of the patients.

The triple negative breast carcinomas have been regarded as the most aggressive over the years. The triple negative cases found to have basal CK5/6 positivity and they are termed as basal subtype and have poorer outcomes[4]. However studies have been done which emphasizes that the positivity of HER2 along with basal marker positivity carries an equally poorer prognosis and hence the basal marker namely CK 5/6 should be included in the panel routinely[5].

HER2 is Human epidermal growth factor receptor 2.Also called as Cluster of differentiation CD 340, Receptor tyrosine kinase Erb 2. It is a member of epidermal growth factor receptor family[6].

HER2 receptor oncogene overexpression on the cell membrane is associated with development and progression of certain aggressive breast malignancies. This overexpression is seen in 20-25% of breast malignancies and it indicates increased rates of recurrence and poor prognosis[8,9]. Targeted therapies are available against HER2 receptor called Trastuzumab, a monoclonal antibody[7].

Cytokeratin 5/6 has a molecular weight of 58KDa and causes diffuse perinuclear cytoplasmic staining. It is expressed in basal subtype of breast cancer which are ER, PR and HER2 negative[10]

In our study, we will evaluate the expression of CK 5/6 and HER2 in Breast malignancies and to determine the prognosis of the disease based on marker positivity. We correlated the expression of the markers with the histological grading.

OBJECTIVE

To study the expression of CK 5/6 and HER 2 in varying grades

of Breast Carcinoma and correlate it with the Nottingham Prognostic Index.

METHODS
The study was conducted in Chengalpattu Medical College and Hospital as a prospective and retrospective record based study among patients who were diagnosed with invasive ductal carcinoma Breast. The study period was 3 years.

Inclusion Criteria:
All patients with invasive ductal carcinoma breast and underwent modified radical mastectomies.

Exclusion Criteria:
Patients who underwent chemotherapy/radiotherapy before the surgery.

Premalignant/Non malignant lesions of breast.

Mesenchymal/Metastatic breast carcinoma.

Institutional ethics committee approval was obtained. A total number of 70 breast cancer cases were selected and biopsy blocks were collected from the Department of Pathology, along with details of the tumor from the register. Hematoxylin eosin staining was done and also immunohistochemistry with CK 5/6 and HER2 were done and analysed. All these samples were graded according to the prescribed Scarff Bloom Richardson Grading.

Statistical Analysis
Statistical Package for Social Sciences (SPSS for Windows V20) was used for data analysis. P value <0.05 were considered for statistical significance to find out the correlation between correlation between the marker expression and grading and NPI scores.

RESULTS
The study was done to find the expression of CK 5/6 and HER 2 in varying grades of Breast Carcinoma and correlate it with the Nottingham Prognostic Index at Chengalpattu Medical College Hospital.

### Table 1- Correlation of HER2 with clinical parameters of the patients

Clinico-pathological parameter			HER2				
		n(70)	EXPERSSION				P-value
			0	1+	2+	3+	
	POST	34	12	2	11	9	
GRADE	I	43	11	2	17	13	0.198
	II	27	12	3	6	6	
NPI	5 and Below	60	20	2	22	16	0.015
	more than 5	10	3	3	1	3	

Pearson Chi-Square

### Table 2-Correlations of CK5/6with Clinico-pathological parameters of the patients

Clinico-pathological parameter			CK5/6			
		n(70)	EXPERSSION			P-value
			Negative	Weak Positive	Strong Positive	
GRADE	I	43	19	11	13	0.339
	II	27	15	8	4	
	III	13	8	4	1	
NPI	5 and Below	60	27	17	16	0.316
	more than 5	10	7	2	1	

Pearson Chi-Square
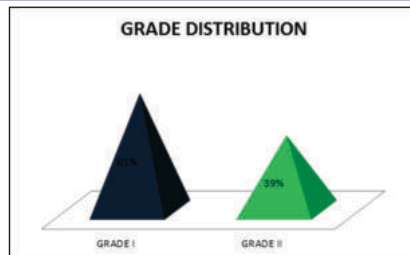
Graph 1: Distribution of Grade of tumors

Of the 70 cases, Grade I tumors were 61 % and Grade II tumors were 39%.
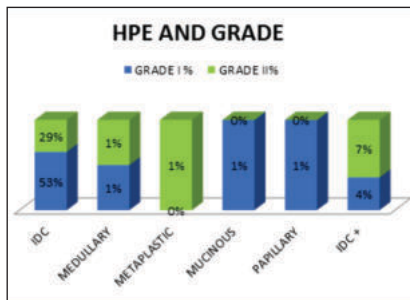
Graph 2: Distribution of HPE type and Grade of tumor

Of the cases, IDC presented as Grade I in 53% and Grade II in 29%. Medullary carcinoma presented as Grade I in 1% and Grade II in 1%. Metaplastic carcinoma presented as Grade II tumor. Both mucinous and papillary carcinoma presented as Grade I lesions in 1% each. IDC variants presented as Grade I tumors in 4% and Grade II lesions in 7%. The correlation of HER 2 and CK 5/6 expression with the Grade of the tumor when analysed individually was not statistically significant(Table 1 and 2)

Nottingham Prognostic Index
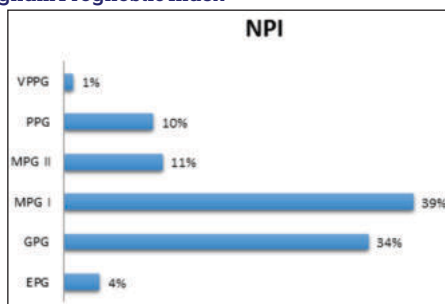
Graph 3: Nottingham Prognostic index of the cases

Out of the 70 cases studied, 1% showed NPI belonging to very poor prognostic group, 10% of the cases belong to Poor prognostic group, 11% of the cases belonged to Moderate prognostic group II, 39% of cases fell under Moderate prognostic group I, 34% of the cases belong to good prognostic category and 4% of the cases were of excellent prognostic group.

Table 3: NPI with Marker expression

NPI GROUPS	CK5/6 and HER2 +	CK5/6 - and HER 2 -	HER 2 + and CK5/6 -	HER 2 – and CK 5/6+
EPG	1	1	1	0
GPG	11	2	7	3
MPG I	9	10	4	4
MPG II	3	2	2	1
PPG	2	4	1	0
VPPG	0	0	0	1

Of the three cases in excellent prognostic group, one case

was CK5/6 and HER 2 positive, one each was CK5/6 negative HER2 negative and HER 2 positive and CK 5/6 negative. Of the Good prognostic group, 11 were CK5/6 and HER2 positive, 2 were CK 5/6 negative and HER2 negative, 7 cases were HER2 positive and CK5/6 negative and three were HER 2 negative and CK 5/6 positive. MPG I- 9 cases were CK5/6 and HER2 positive, 10 cases were CK5/6 and HER 2 negative, 4 cases each were HER2 positive CK5/6 negative and HER 2 negative and CK 5/6 positive.MPG II-3 cases were CK 5/6 and HER2 positive, 2 cases each were HER2 and CK5/6 negative, HER 2 positive and CK5/6 negative and one case was CK 5/6 positive and HER 2 negative. Poor prognostic group-2 cases were CK5/6 and HER2 positive, 4 cases were CK5/6 and HER2 negative, 1 case was CK5/6 negative and HER2 positive. Very poor prognostic group- one case was CK5/6 positive and HER2 negative.

Table 4: Correlation of Grade and Marker status

GRADE	CK5/6 and HER 2 -, CK 5/6+ and HER2 -,CK5/6 – and HER 2 +	CK 5/6and HER 2 +	P value
GRADE I	24	19	0.167
GRADE II	20	7	0.314
TOTAL	44	26	

Of the 70 cases, Grade I tumors with marker positivity in both CK 5/6 and HER 2 were 19,while grade II tumors the marker positivity of CK 5/6 and HER2 were seen in 7 cases.

And correlation of CK 5/6 – and HER 2 -, CK5/6 + with HER 2 – and HER 2 + and CK 5/6 negative was done with CK5/6 and HER 2 positivity in grade I lesion and it was not statistically significant(p value-0.167).

And correlation of Grade II tumors with CK 5/6 and HER 2 positive tumors with other categories showed there was no statistical significance (0.314).

NPI and Marker positivity

Table 5:Correlation of NPI and Marker positivity

Marker	EPG	GPG	MPG I	MPG II	PPG	VPPG	TOTAL	P value
CK5/6 and HER 2 +	1	11	9	3	2	0	26	.762
Others	2	13	18	5	5	1	44	

NPI was taken as the continuous variable and CK 5/6 and HER 2 expressions was grouped as those with with HER 2 and CK 5/6 positive as one group and it was compared with CK 5/6 and HER2 negative, CK 5/6- and HER+ and CK 5/6 + and HER2 negative. The P value was not statistically significant (.762).
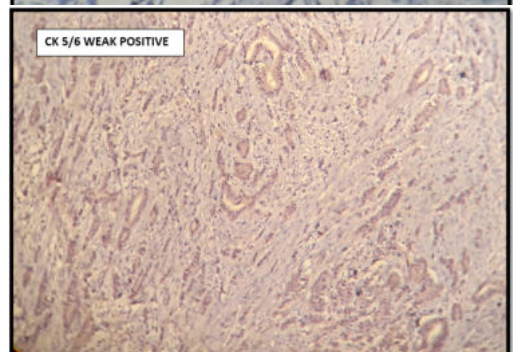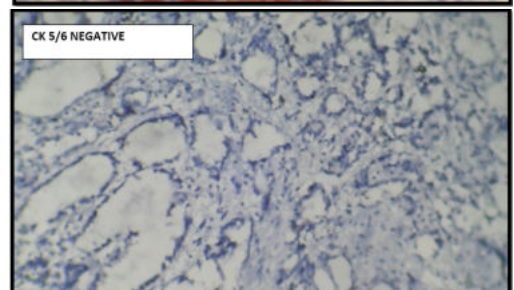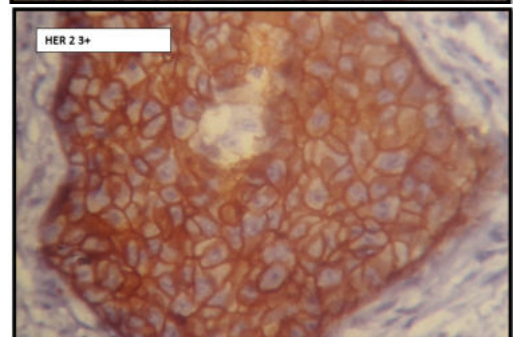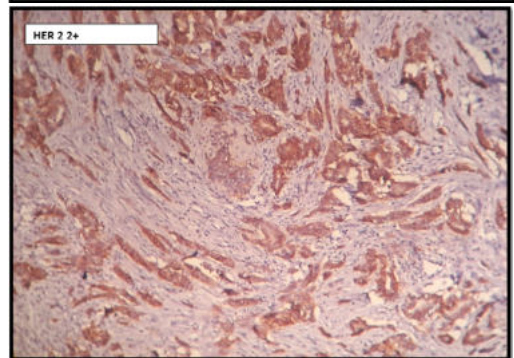
MEAN NPI BASED ON MARKER STATUS
Table 6: Mean NPI based on Marker status

HER 2	CK 5/6	MEAN NPI
Positive	Positive	3.58
Positive	Negative	3.53
Negative	Positive	3.95
Negative	Negative	4.17

In our study, The mean NPI of HER2 and CK 5/6 positive cases were almost similar to that of HER2 positive and CK 5/6 negative cases. The HER2 negative cases with the either CK 5/6 positive or negative had higher Mean NPI and the p value calculated when comparing NPI as the dependent variable using Two way Anova was also statistically insignificant(.638)

Table 7: CK5/6 and NPI groups

NPI GROUPS	CK5/6 +	CK5/6 -
EPG	1	2
GPG	14	9
MPG I	13	14
MPG II	4	4
PPG	2	5
VPPG	1	0

Most of the CK5/6+ cases,20 cases were of the Moderate prognostic groups, Poor prognostic group and very poor prognostic group.

CK 5/6 strong positive
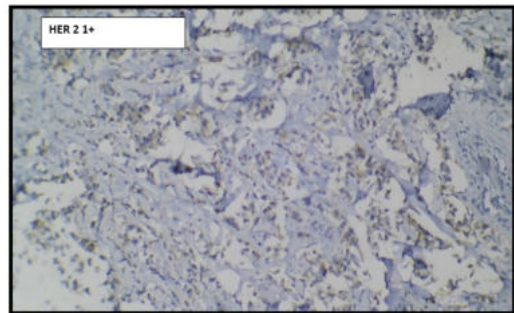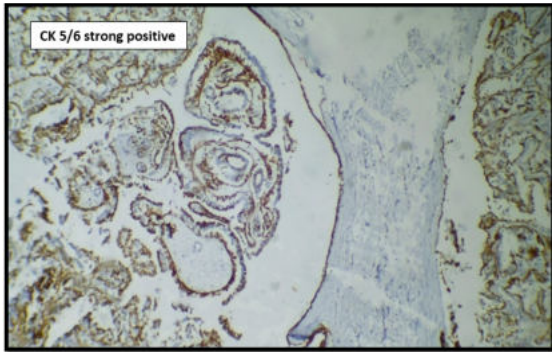
DISCUSSION
Comparison of HER 2 with Grade
*   In our study of the 70 cases, 43 cases were Grade I and 27 cases were grade II.
*   Of the 43 cases of grade I, 13 were negative for HER2, 17 cases were 2+ positive and 13 cases were 3+ positive. Grade II- 15 were negative, 6 cases each showed 2+ and 3+ respectively.
*   Praveen Adusumili et al had studied 212 positive cases of HER 2 of which 108 cases were Grade I, 63 cases were Grade II and 41 cases were Grade III[11].
*   Houber et al study shows that out of 8096 ductal malignancy cases, 950 cases were Grade I, 3276 cases were Grade II, 3053 cases were Grade III[12].
*   Kalal Iravathy Goud et al had studied 90 cases, 2 grade I tumors were negative for HER 2,out of 20 Grade II tumors, 12 were negative 5 3+ positive and 3 were 2+. Out of 68 grade III tumors, 40 were 3+positive, 19 were negative and 9 were 2+ [13].

HER 2 positivity in Grade I and Grade II tumors shows similar results as the above mentioned articles

Comparison of CK 5/6 with Grade
Out of 43 cases of grade I, 19 cases were negative, 11 were weak positive and 13 were strong positive. Out of the 27 grade II cases, 15 were negative, 8 cases were weak positive and 4 cases were strong positive.
*   According to Chandrika Rao et al study of 50 cases of triple negative breast cancers, Grade II tumors were 12 of which 4 cases were CK 5/6 positive,8 cases were CK 5/6 negative and Grade III tumors were 38,16 cases were CK 5/6 positive and 22 cases were CK5/6 negative and the P value correlation with grade and CK 5/6 status was statistically insignificant(>0.05) [14].
*   Neelam Sood et al study on basal marker expression in triple negative breast cancers included 36 cases, Grade I tumors were 5, Grade II were 17 and Grade III were 14 and there was a positive correlation between Grade and CK 5 expression(P value -0.052) [15].

Compared with articles, our study has similar results for CK 5/6 expression with grade.

Comparison of Grade and HER2 and CK 5/6 positive status
HER2 and CK5/6 positve cases in Grade I were 19 cases and Grade II cases were 7 cases and of the 26 cases which showed CK 5/6 and HER 2 positive, 13 showed strong CK positive along with HER2 positivity, 10 cases were of Grade I and 3 cases were grade II. CK 5/6+ and HER 2+ cases were compared with other groups of marker positivity for Grade I and Grade II and found to be statistically insignificant(p values-0.167, 0.314).

*   Begoña Martin-Castillo et al studied the HER2 and CK5/6 positive tumors and has classified CK 5/6 weak positive and HER 2 positive as Basal1-HER2+ tumors prognostically similar to HER2+ tumors,CK5/6 strong positive along with HER2 positive as clinically aggressive basal 2 -HER2+ which will be likely to be unresponsive to Transtuzumab[16].

According to the this categorization, our cases fall into

Table 8: Basal HER 2 distribution

Basal 1- HER 2 +	Basal 2- HER2+
13	13

*   Begoña Martin-Castillo et al studied the HER2 and CK positive tumors and compared it with the grade of the tumor, 5 cases with Grade II and 14 cases were grade III and found that the P value was found to be statistically not significant(P value-0.934) (16).

Comparison of HER2 with NPI
Of the 60 cases with NPI less than 5, 22 cases (20+2) were negative, 22 cases were 2+ and 16 cases were 3+. Of the 10 cases with NPI greater than 5, 6(3+3) were negative, 3 were 2+ and 1 case was strong 3+ positive. The p value (0.015) was found to be statistically significant.

Comparison of CK 5/6 with NPI
Of the 60 cases with NPI less than 5, 27 were negative, 17 were weak positive and 7 were strong positive. Of the 10 cases with NPI more than 7 were negative, 2 were weak positive and 1 was strong positive. But the p value (0.316) was not statistically significant.

Comparison of marker positivity with NPI groups
Of the 26 cases which were CK5/6 and HER2 +, 1 belonged to excellent prognostic group, 11 cases were of Good prognostic group, 9 cases belonged to moderate prognostic group I, 3 belonged to moderate prognostic group II and 2 cases were of poor prognostic group and HER2 +CK5/6-, HER2 CK5/6 – and CK5/6 HER2 – were totally 44 cases, of which 2 were excellent prognostic group, 13 were good prognostic group, 18 cases were moderate prognostic group I, 5 cases were moderate prognostic group II, 5 cases were of poor prognostic group and 1 case was of very poor prognostic group. The p value(0.762) comparing the CK5/6+HER2+ group with others showed no statistical significance in relation with NPI.

Most of the cases in our study most of the cases, 35 cases were of moderate prognostic group.

Mean NPI with Marker status
The mean NPI for CK5/6 and HER 2 positive cases was 3.58, for HER2 positive and CK 5/6 negative cases was 3.53, for HER 2 negative and CK 5/6 positive cases was 3.95 and for HER 2 negative and CK 5/6 negative cases was 4.17.

*   Begoña Martin-Castillo et al in their study comparing HER2 and CK 5/6 positive cases with other HER + cases compared the adjuvant chemotherapy using Trastuzumab and its response and local recurrence and found that the local recurrence rates were statistically significant when comparing with the HER2 and CK 5/6 positivity (p value-0.007)

Though statistical significance couldn't be proven, NPI was used to assess the prognosis in our study since the follow up of the patients and the treatment details were not available.

Basal 2-HER 2 cases (HER2 + with strong CK 5/6+) belonging to Grade II tumors mostly had Higher NPI, those cases belonging to Grade I were all in the Good prognostic group and hence it becomes necessary to follow up these patients and subject them to more aggressive treatment options based on CK 5/6 positive status in accordance with the study by Begoña Martin-Castillo et al[16].

SUMMARY AND CONCLUSION

Breast carcinoma is the most commonly occurring malignancy in women world wide and accounts for significant morbidity and mortality, hence extensive studies are essential to make sure that treatment modalities available are put to maximum use at the earliest to curb the disease burden.

Objective of this study was to evaluate the Expression of CK 5/6 and HER2 in Breast malignancies, to determine the prognosis of the disease based on marker positivity, to determine the prognosis of the disease with Nottingham's Prognostic index, correlate the expression of the markers with the histological grading, and the NPI types and to categorise Basal HER2 positive tumors as Basal 1-HER2 and Basal 2-HER2.

A total of 70 cases proven as invasive breast carcinoma histopathologically were studied retrospectively and prospectively in Chengalpattu Government Medical College for a period of 3 years. The Most of the cases were histopathologically diagnosed as Infiltrating Ductal carcinoma NOS and most of the cases were belonging to Scarff Bloom Richardson grade I. The cases were grouped into Nottingham's Prognostic groups based on the size, grade and lymph node status and in our study most of the cases fell into the moderate prognostic group with the 10 year survival rates between 70-80%. Immunohistochemistry was done on the paraffin embedded tissue sections and expression of HER2 and CK 5/6 was documented and analysed.

Based on the percentage of cells expressing HER2 membrane staining it was scored as negative, 1+ which were considered negative and 2+ and 3+ which were considered positive. CK 5/6 showed cystoplasmic staining and was scored as negative, weak positive and strong positive.

The correlation of NPI with the HER2 expression was found to be statistically significant.

The marker positivity of CK5/6 and HER2 were analysed and statistical correlation was done with other groups taking Grade and NPI as dependent variables. Though statistically not significant, the CK 5/6 strong positive tumors along with HER positivity was quite higher in Grade I (10 cases) compared to the 3 cases in Grade II. With studies available indicating that the CK 5/6 strong positive expression along with HER 2 positivity, showing resistance to Trastuzumab therapy. Our study population who show strong CK5/6 positivity in Good prognostic group also need to be followed up and treated with aggressive therapy like that of Moderate prognostic group and Poor prognostic group even though the Grade and NPI is low. Hence this underlines the fact that Basal 2- HER2 subtypes should be given more importance and Immunohistochemical analysis is a must even in cases of lower histopathological grades and NPI scores.

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