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STUDY OF EXPRESSION OF CK-5/6 AND EGFR -1 IN TRIPLE NEGATIVE BREAST CANCER IN CORRELATION WITH EXPRESSION OF Ki67

Oncopathology

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ABSTRACT

Context: Breast carcinoma is the most common malignant tumour and the leading cause of cancer deaths in women. Based on the gene expressing profiles, molecular classification of breast cancer is divided into five subtypes: luminal-A, luminal-B, triple negative, normal breast–like and Her-2neu positive. Basal-like and tripe negative are not synonymous. This subtype confers a markedly poor prognosis and lacks the benefit of a specific therapy. **Aims:** To study the expression of CK5/6 and EGFR-1 basal markers in triple negative breast cancers(TNBC), to classify triple negative breast cancer in basal and non-basal types and Ki-67 expression correlation. **Methods and Material:** The present study includes 49 triple negative breast cancer cases over a 6 year period (August 2011-July 2017). These cases were classified into basal and non-basal types of triple negative cancers based on basal markers(CK-5/6 and EGFR-1). Clinical, pathological features like size, grade, type and lymphnode status were studied and correlated with Ki-67 expression. **Statistical Analysis Used:** Pearson chi-square test was used for statistical correlation. **Results:** Triple negative breast cancer cases were older age(52.5years), with adverse pathological characteristics of a high tumour size, high grade with most common histological subtype-IDC, lymphnode metastasis. 41 cases(84%) showed expression of basal markers (CK-5/6 and/or EGFR-1). 30/49 cases showed high Ki-67 expression. **Conclusions:** Basal type of TNBCs correlation with size, histological grade and showed lymphnode metastasis and Ki-67 expression was statistically significant. Basal phenotypes have a more aggressive course than non-basal phenotype. Majority of cases (41/49 cases) showed EGFR-1 expression. Therefore TNBCs could potentially benefit from EGFR-targeted therapeutic strategies.

KEYWORDS

Triple-Negative Breast Cancer; Basal Type; EGFR-1; CK-5/6; Ki-67

INTRODUCTION

Breast carcinoma is the most common malignant tumour and the leading cause of cancer death in women.[1] It has ranked number one cancer among Indian females with age adjusted rate as high as 25.8 and mortality 12.7 per 100,000 women.[2]

Patients with triple-negative breast cancer (TNBC) derive no benefit from molecularly targeted treatments, such as endocrine therapy, as they lack the appropriate targets for these drugs.[3]

TNBC is characterized by its biological aggressiveness and poor prognosis, consisting of two subtypes, basal and non-basal. Basal type was defined as CK-5/6 positive and/or EGFR-1 positive and non-basal type was defined as having no expression of these two markers. Ki-67, another marker being studied for its implication if any.

So this study was undertaken to study expression of CK5/6 and EGFR-1 basal markers in triple negative breast cancer and its correlation with clinicopathological and Ki 67 expression as prognostic index.

SUBJECTS AND METHODS:

Patients And Samples:

The study cohort comprised of 49 cases of all triple negative surgical specimens from patients operated for breast carcinoma of all ages received for routine histopathological evaluation.The duration of this was 6 years (1st August 2011 to 31st July 2017).

Of the total 49 cases studied 37 were modified radical mastectomies, 09 simple mastectomies and 3 lumpectomies. For all the cases lymph nodes were received. In modified radical mastectomy cases, axillary lymph node dissection was done in all cases and also lymph nodes were identified in the axillary tail in 27 cases. In simple mastectomy cases, lymph nodes were identified in the axillary tail while lumpectomy cases were followed by sentinel lymph node biopsy.

The paramaters collected include age, sex, menstrual status, site and tumour size on gross examination

Specimens were subsequently formalin fixed, paraffin embedded and stained by haematoxylin and eosin (H & E) for histopathological study to assess histological subtype of Breast cancer, histological grading of tumour, axillary nodal status, lymphovascular invasion and any other significant features.

Histological grading of tumour was done according to Modified Bloom Richardson grading and staging according to TNM Classification designated by American Joint Committee on Cancer(AJCC).

Immunohistochemistry:

Immunohistochemistry was done for EGFR-1, CK - 5/6 and Ki – 67.

The immunostained slides were examined for membranous staining in case of EGFR -1. It was assessed for EGFR according to DAKO criteria.

· When stain is faint and incomplete membrane positivity- 1+/ weak.
· When stain is moderate and complete/incomplete membrane positivity - 2+/ moderate.
· When stain is Strong and complete membrane positivity – 3+/ strong.

The immunostained slides were also examined for membranous or cytoplasmic staining incase of CK – 5/6 and nuclear staining in case of Ki – 67.

Ki-67 was scored as percentage of positively stained cells among the total number of malignant cells and divided into 2 groups –
· Low (<10%)
· High (≥10%)
· <1% considered as negative.

Statistical Analysis:

The relationship between various parameters such as age, menopausal status, tumour size, tumour extent, histologic type, histologic grade, and lymph node status, the expression of EGFR-1, CK-5/6 and Ki-67 were studied. Based on their expression classified into basal and non-basal type.

The statistical analysis for correlation among these parameters was determined using the Pearson chi-square test. Significance was assumed at p-value less than 0.05.

RESULTS:

In the present study, 49 cases of triple negative breast cancer were evaluated clinicopathologically and subsequently evaluated for IHC markers EGFR-1, CK 5/6 and Ki-67. The clinicopathological parameters represented in table 1.

Table 1: Clinicopathological characteristics of Triple-negative

breast cancer (ER negative, PR negative and HER-2 negative). Abbreviations – IDC – Infiltrating ductal carcinoma no special type, DCIS – Ductal carcinoma in situ.

Clinicopathological variables	Frequency (percentage) N=49
Age(years) mean	**52.5yrs**
<40	10(20)
41-50	16(33)
>50	23(47)
Tumor size (cm)	
<2	7(14)
2-5	15(31)
>5	27(55)
Menopausal status	
Premenopausal	19(39)
Post menopausal	30(61)
Side of affected breast	
Left	28(57)
Right	21(43)
Histopathological diagnosis	
IDC	43(88)
Medullary carcinoma	5(10)
Papillary carcinoma	1(2)
TNM staging	
Stage I	4(8)
Stage IIA	13(27)
Stage IIB	15(31)
Stage IIIA	9(18)
Stage IIIB	8(16)
Tumor grade	
Grade I	9(18)
Grade II	12(25)
Grade III	28(57)
Lymphovascular invasion	
Present	12(24)
Absent	37(76)
Lymph node metastasis	
Present	22(45)
Absent	27(55)
Associated histological features	
Desmoplasia	18 (36.7)
Necrosis	14(28.5)
Lymphocytic infiltrate	8(16.3)
Adipose tissue infiltrate	5(10.2)
Elastosis	4(8.2)
Fibrocystic change	3(6.1)
DCIS	2(4.1)

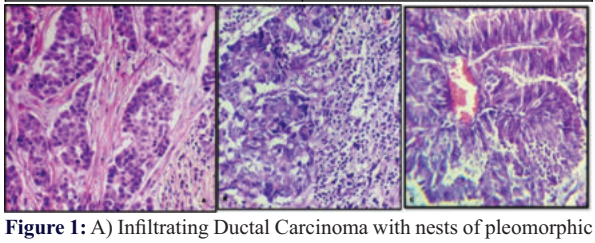

Figure 1: A) Infiltrating Ductal Carcinoma with nests of pleomorphic cells having prominent nucleoli and occasional mitosis [H&E 400x], B) Medullary Carcinoma showing sheets of cells with syncytial growth pattern [H&E 400x]and C) Papillary carcinoma showing neoplastic epithelial cells with hyperchromatic nucleus lining a papillary frond.[H&E 400x]

Among the 49 triple negative breast cancers, 22cases (36.67%) were retrospective and 38cases (63.33%) were prospective. The mean age was 52.5years (range 21-85years), with majority of the patients being >50yrs (47%), most of the patients being menopausal 30cases (61%) and left breast was predominantly affected 28 cases (57). The tumour size ranged from 1 to 15 cms with mean of 4.52cms. 27 cases (55%) measured >5cms followed by 15 cases (31%) between 2-5cms and 7 cases (14%) <2cms. Most common histological type found was 43 cases (88%) of invasive ductal carcinoma, followed by 5 cases (10%) of medullary carcinoma and 1 (2%) case of papillary carcinoma (Figure 1).Nottingham modification of the Bloom Richardson system was followed for histological grading. Majority of the cases were grade III constituting 57%(28 cases) followed by 25% (12 cases) of grade II and 18% (9 cases) of grade I. Out of the 49 cases, 28 cases were found to be stage II (57%), 17 cases (35%) were in stage III and 4 (8%) in stage I. Lymphovascular invasion (LV) was noted in 12 cases (24%) and absent in 37 cases (76%). Lymph node metastasis were noted in 22 cases (41%). Majority of the cases (27cases; 59%) did not show metastasis in the lymph nodes.Desmoplasia was the most common associated histological finding in the breast carcinoma cases, observed in 18 cases (36.7%). Other findings include necrosis in 14cases (28.5%), Lymphocytic infiltrate in 8 cases (16.3%), adipose tissue infiltrate in 5 cases (10.2%), elastosis in 4 cases (8.2%), fibrocystitic disease in 3 cases(6.1%) and ductal carcinoma in-situ (DCIS) in 2 cases (4.1%).

IMMUNOHISTOCHEMISTRY RESULTS

The frequency of basal markers expression was assessed. Out of 49, 41 cases were positive for either CK-5/6 and /or EGFR-1 positive that is basal type (Table 2) (Figure 2 and 3). Majority of the cases were of high Ki-67 index (30 cases; 61%) and 19 cases (39%) were of low proliferative index (Table 3).

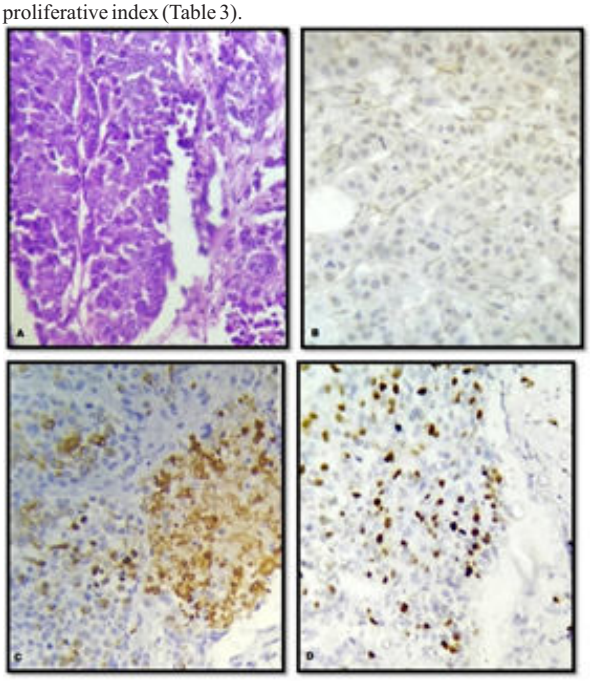

Figure 2: Basal type . A) IDC H&E (400X), B) EGFR-1 positivity (weak), C) CK-5/6 positivity and D) Ki-67 showing > 10% (75%)[400x]

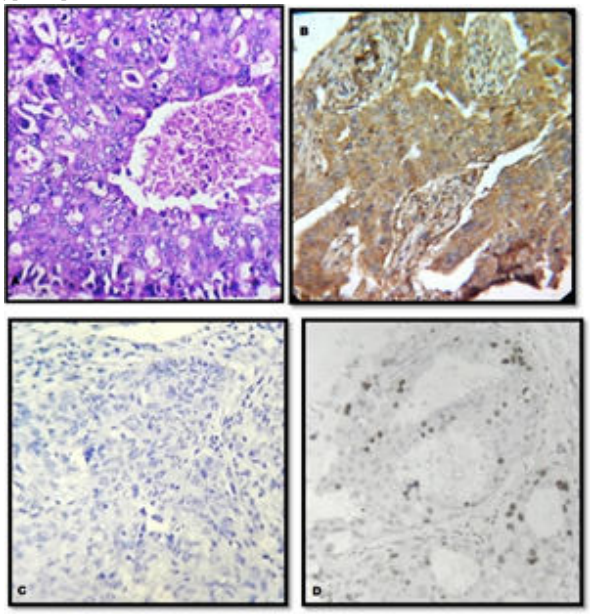

Figure 3: Basal type. A) IDC H&E(400X), B) EGFR-1 positivity

(strong), C)CK-5/6 negativity and D) Ki-67 showing >10% (30%).

Table 2: Expression of EGFR-1 and CK -5/6 in triple negative breast cancers.

IHC panel	
CK5/6 + EGFR-1 +	6 (14%)
CK5/6 + EGFR-1 -	0 (0)
CK5/6 - EGFR-1 +	35 (69%)
CK5/6 - EGFR-1 -	08 (17%)

Table 3: Ki 67 Expressions in Triple Negative Breast Cancer

Ki 67	N=49 (%)
< 10%	19(39%)
10%	30(61%)

Triple negative breast cancer cases were associated with adverse pathological characteristics of a high tumour size (p = 0.01), high grade (p = 0.02) with most common histological subtype- infiltrating ductal carcinoma (IDC) (p = 0.019), lymph node metastasis (p = 0.04) (Figure 4).

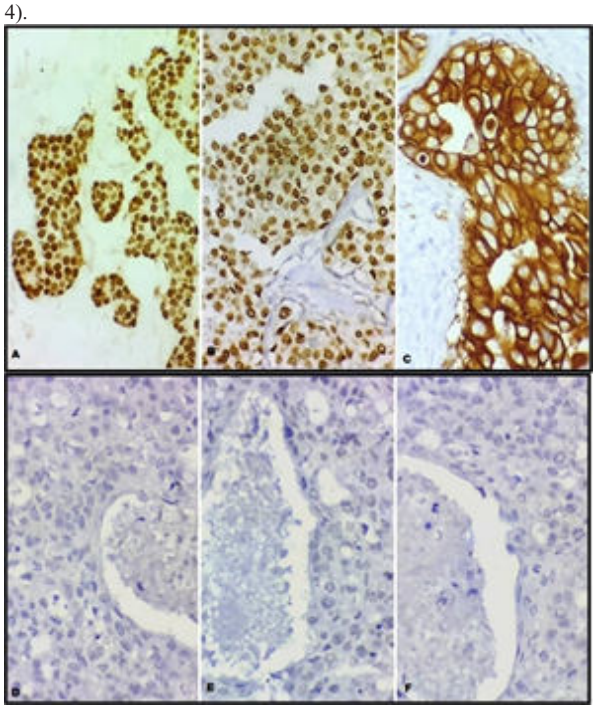

Figure 4: Control: A) ER positivity, B) PR positivity andC) HER2/neu positivity [400x]; Triple Negative subtype – D) ER negativity, E) PR negativity and F) HER2/neu negativity [400x]

Basal type of triple negative breast cancers were significantly larger size (>5.1cm), associated with IDC typeand weremainly of high grade (63%).

Most of cases (21cases; 51%) basal type of triple negative breast cancer were showing lymph node metastasis. Though there was no significant association of LV emboli with basal type of triple negative breast cancers (Table 4). Basal markers were not significantly associated with TNM staging of triple negative breast cancer (Table 4). Majority of the cases were of high Ki-67 index (30 cases; 61%) and 19 cases (39%) were of low proliferative index (Table 5).

Table 4: Relationship Of Basal Markers With Tumor Size, Histology, Grading, Lymphovascular Emboli, Lymphnode Status And TNM Staging.

	Basal markers Either of EGFR-1 OR CK5/6 +.		EGFR 1		CK 5/6	
	Basal type	Non basal type	Positive	Negative	Positive	Negative
	n=41(%)	n=8(%)	n=41 (%)	n=8(%)	n=6(%)	n=43 (%)
Tumor sizeCms						
<2	07 (17%)	00	07 (17%)	00	0(00%)	7(16%)
2.1-5	09(22%)	6 (75%)	09(22%)	6 (75%)	4(67%)	11(26%)
>5.1	25(61%)	2 (25%)	25(61%)	2 (25%)	2(33%)	25(58%)
Histology						
IDC	38 (93%)	05 (62.5%)	38 (93%)	05 (62.5%)	06 (100%)	37 (86%)
Medullar	03 (07%)	02(25%)	03 (07%)	02(25%	00	05 (12%)
Papillary	00(00%)	01 (12.5%)	00(00%)	01 (12.5%)	00	01 (02%)
Grading						
Grade I	08 (20%)	1 (12.5%)	08 (20%)	1 (12.5%)	1 (17%)	8(19%)
Grade II	07 (17%)	2(25%)	07 (17%)	2(25%)	3(50%)	6(14%)
Grade III	26 (63%)	5 (62.5%)	26 (63%)	5 (62.5%)	2 (33%)	29(67%)
LV Emboli						
Present	12 (29%)	00	12 (29%)	00	03 (50%)	09 (21%)
Absent	29 (71%)	08 (100%)	29 (71%)	08 (100%)	03 (50%)	34 (79%)
Lymph Node Status						
Present	21 (51%)	01 (12.5%)	21 (51%)	01 (12.5%)	03 (50%)	19 (44%)
Absent	20 (49%)	07 (87.5%)	20 (49%)	07 (87.5%)	03 (50%)	24 (56%)
TNM						
I.	04	00	04 (9.7%)	00	00 (0)	04 (09%)
II. A	08 (19.5%)	05 (62.5%)	08 (19.5%)	05 (62.5%)	02 (33.3%)	10(23%)
II. B	13 (31.7%)	02 (25%)	13 (31.7%)	02 (25%)	02 (33.3%)	14 (33%)
III. A	08 (19.5%)	01 (12.5%)	08 (19.5%)	01 (12.5%)	01 (16.7%)	08 (19%)
III. B	08 (19.7%)	00	08 (19.7%)	00	01 (16.7%)	07 (16%)

Table 5: Relationship Of Basal Markers With Ki 67 Expression

Ki 67	Basal markers Either of EGFR-1 OR CK5/6 +		EGFR 1		CK 5/6	
	Basal type	Non basal type	Positive	Negative	Positive	Negative
	n=41 (%)	n = 8 (%)	n= 41 (%)	n = 8(%)	n=6 (%)	n=43 (%)
< 10%	12 (29%)	07(87.5 %)	12 (29%)	07 (87.5%)	4 (66.6%)	15 (35%)
10%	29 (71%)	01 (12.5%)	29 (71%)	01 (12.5%)	2 (33.3%)	28 (65%)

DISCUSSION:

As breast cancer shows remarkable heterogeneity therefore the need arose for recent classification of breast cancer into subgroups based on the gene expression profile. Based on the study of these profiles, breast cancer can be divided into five subtypes: luminal A, luminal B, triple negative, normal like and HER2 positive subtype.[3,4]

Triple-negative breast cancer is a state where there is absence of estrogen receptor (ER), progesterone receptor (PgR) and human epidermal growth factor 2 (HER2) expressions. This is characterized by biological aggressiveness and poor prognosis. This further consists of two subtypes – basal and nonbasal.[5]TNBC and basal type are never synonymous instead basal type has similar behaviour as TNBC.[6]

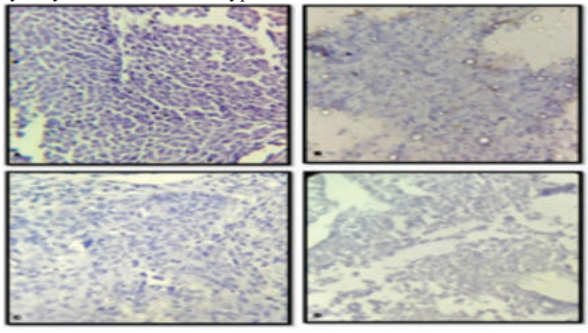

Figure 5: Non Basal type. A) IDC H&E (400X), B) EGFR-1 (negativity), C) CK-5/6 negativity and D) Ki-67 : negative (<10%)

Basal type is CK-5/6 - positive and/or EGFR–1 positive, whereas non-basal type is having o expression of these two markers[5](Figure 5). Basal type TNBC has high histological grade, p53 mutation, usually expresses basal cytokeratin (CK-5/6, CK-14 and CK-17), epidermal growth factor receptor (EGFR) overexpression, significantly associated with Ki-67 labelling index, p53 expression and BRCA1 expression, and showed a shorter overall survival than non-basal type.[7]

The present study includes 49 triple negative breast cancers which were classified into basal and non-basal subtypes based on the expression of basal markers.

In the present study, basal type of TNBC cases were of older age group i.e., >51years and majority were post-menopausal status that correlate well with Rakhaet al[7] and Choccalingam et al.[8]

The commonest tumour size was more than 5cm, as observed by Thikeeet al[9]

CONCLUSION:

Breast cancer is a global disease with rising incidence in Indian women.

Triple-Negative Breast Cancer (ER-negative, PR-negative, Her2neu-negative) encompasses a heterogeneous group of tumours that possess distinctive pathological and clinical features. However, basal-like carcinomas are not synonymous with triple negative carcinomas.

TNBC can be sub classified using positive markers CK 5/6 and EGFR-1 and significantly worse outcome group can be identified among the triple-negative cases. Basal phenotypes have a more aggressive course than non-basal phenotype.

In present study of 49 triple negative breast cancer cases, most (84%) were of EGFR-1 over- expression alone. Majority (84%) were of basal type of triple breast cancer which was seen postmenopausal patients. Basal type of triple negative cases was associated with larger size of tumour, higher grade, IDC sub type and lymph node metastasis and these parameters were statistically significant.

However high Ki-67 index was associated with basal type and it found to be statistically significant.

Inconsideration of the fact that the prognosis is clearly different between basal type and non-basal type, it is evident that it is necessary to distinguish between these two types and establish different therapeutic strategies for them.

In the future, it will be best to treat triple negative breast cancer, especially basal type, by neo-adjuvant chemotherapy, and it will be desirable to establish effective drug treatment methods such as high-dose chemotherapy and/or EGFR-1 targeted therapy.

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