Research article

An insight into immunohistochemical profile of triple negative breast carcinoma in a tertiary care center

M. Lauvanya[1], G. Bhuvana[2], K. Swaminathan[3], M. Subarathi[4]

[1]Department of Pathology, Kanyakumari Government Medical College, Asaripallam, Tamil Nadu, India
[2]Department of Pathology, Government Medical College, Dindigul, Tamil Nadu, India
[3]Department of Pathology, Government Medical College, Tirunelveli, Tamil Nadu, India
[4]Department of Biochemistry, Madurai Medical College, Madurai, Tamil Nadu, India

(Received: December 2023 Revised: January 2024 Accepted: February 2024)

Corresponding author: **M. Subarathi.** Email: drsubarathi@gmail.com

ABSTRACT

Introduction: Triple Negative Breast Cancer (TNBC) is a type of breast cancer that fails to express Estrogen Receptor (ER), Progesterone Receptor (PR) and Human Epidermal Growth Factor Receptor 2 (HER2) in Immunohistochemical analysis. TNBC has a unique molecular profile, aggressive nature and lack of efficient targeted therapies. The aim of the study was to evaluate the expression of basal markers like Epidermal Growth Factor Receptor (EGFR) and Cytokeratin 5/6 (CK 5/6) and to correlate their expression with the clinico-pathological parameters, thus enabling the identification of the aggressive basal like phenotype of TNBC in resource-constrained settings.

Methodology: The study was conducted at a South Indian tertiary care centre for 2 years. Data such as age of presentation, tumour measurements, margins and necrosis, numbers, levels and sizes of lymph nodes were collected. Data on histological type and grade, *in situ* component, lymphovascular and perineural invasion, stromal reaction and lymph node status were recorded. Primary Immunohistochemical studies were performed for the markers ER, PR and HER2.Patients with negative expression for all the three markers were identified. IHC studies for EGFR and Cytokeratin 5/6(CK5/6) were done on tissue blocks and the expression of these two markers were equated with the clinico-pathological parameters.

Results: Among the 94 cases, 44 cases (46.8%) expressed CK5/6 and 46 (48.9%) expressed EGFR. A plausible association was noted between the expression of the two markers with age of the patient, tumour size, histological grade and tumour necrosis.

Conclusion: Combination of the time honoured gross and histopathological examinations along with the application of basal markers like CK 5/6 and EGFR, helps in identifying the basal-like phenotype with dismal clinical outcome among the triple-negative breast cancers in resource-constrained settings. An insight into such an aggressive tumour, provided by these markers may be critical in designing personalized treatment strategies for such patients.

Keywords: Breast cancer; triple-negative; CK5/6; EGFR.

INTRODUCTION

Malignancies bring about a major havoc globally. The most frequent malignancy encountered in women is Breast carcinoma, with 2.3 million cases and 685 000 deaths worldwide in 2020(1).In India,13.5% of all cancer cases and 10.6% of all deaths were attributable to carcinoma breast(2),with roughly about 60% of the cases being in stage III or IV of the disease at the time of diagnosis(3).It is a multifaceted and diverse illness that includes a range of entities with unique morphological features, immunohistochemistry profiles, and biological characteristics.

Breast tumours are categorised into distinct groups based on the expression of the proteins ER and HER2 as: Luminal Cancers that are positive for ER and negative for HER2, HER2 cancers, that overexpress HER2 and an unique type known as triple negative breast cancers (TNBC),that are identified by the lack of expression of the Estrogen Receptor (ER), Progesterone Receptor(PR)and Human Epidermal Growth Factor Receptor 2 (HER2)(4,5).Aggressive histomorphology,BRCA1 association, resistance to hormonal treatment, a reduced survival rate, and a dismal prognosis characterise this type of cancer(5).

Basal like molecular subtype of TNBC is distinguished by a high histological grade, Basal Cytokeratin expression (CK5/6, CK14, and CK17) in most cases, Epidermal Growth Factor Receptor (EGFR) overexpression, p53 mutation and a shorter survival than non-basal type(5).In this investigation, we attempted to determine the proportion of Basal type TNBC in our cases by employing five immunohistochemistry markers: ER, PR, HER2, Cytokeratin 5/6, and EGFR, which recognizes basal-like TNBC with 100% specificity and 76% sensitivity (6).

MATERIALS AND METHODS

The research was carried out for a duration of two years at the Department of Pathology, Tirunelveli Medical College Hospital in Tamil Nadu, after the clearance from the Institutional Ethics committee. On the grounds of ER, PR and HER-2 negativity, 94 women with triple-negative primary invasive breast carcinoma were chosen after acquiring a written informed consent.

Criteria for inclusion and exclusion

All histologically confirmed cases of primary breast cancer that tested negative for Estrogen receptor, Progesterone receptor, and Her-2 neu receptor were considered. Positive cases were not included in the analysis. A structured questionnaire was used to elicit information from respondents, regarding their basic demographic data, presenting illness with duration, menstrual, marital and lactational history, family history of malignancies and relevant clinical findings and investigations.

Modified radical mastectomy samples of these patients fixed in 10% neutral buffered formalin, were examined grossly based on standard protocols and relevant details about the tumour such as location, size, nipple and areola status, condition of overlying skin, distance from resection margins, existence of tumour necrosis, and information about the lymph nodes were documented.

Tissue sections measuring 4 to 5 µm were stained with hematoxylin and eosin stain, reported, graded using the Nottingham modification of the Scarff–Bloom–Richardson system (7). The histological type and grade of the tumour, nipple and areola status, overlying skin status, surgical margins, in situ component, lymphovascular and perineural invasion, stromal response, stromal elastosis and data about the lymph nodes were recorded.

Based on the lymphocytic aggregates within the tumour, peritumoral lymphocytic infiltrates were classified (8) as mild (<1/3rd of the tumour shows lymphocytic infiltrates), moderate (1/3rd to 2/3rd of tumour shows lymphocytic infiltrates) and marked (>2/3rd of tumour shows lymphocytic infiltrates) respectively.

CK 5/6 (EP 24/EP 67 clone) and EGFR (EP 22 clone) rabbit monoclonal antibodies were employed in the Immuno-histochemical study in accordance with standard protocols. Polyexcel horse radish peroxidase polymer was applied as the secondary antibody. The diamino-benzidine tetrachloride (DAB) method was used for colorimetric detection of antigen-antibody reactions. EGFR positivity was observed in the cytoplasm and membrane of the tumour cells and cytoplasmic positivity was noted with CK 5/6.Positive tissue control was employed to ensure proper functioning of reagents. Brown staining, which results from the DAB chromogen labeling the antigen-antibody combination at the target antigen site, validated the positive reaction. The test specimen results were deemed invalid if the positive control tissues did not exhibit the anticipated staining pattern.

SPSS was used to input and scrutinize the data. The frequencies and percentages of all the variables were calculated. The Pearson Chi-square test was utilized to investigate the relationship between EGFR and CK5/6 expressions and clinicopathological features of the tumour. Results were considered statistically significant if the p-value was <0.05.

RESULTS

The study group included 94 patients who were ER, PR and Her2 neu negative. These patients were in an age range of 25 and 84 years (mean 50.4 years) and 38 patients (40.4%) above 50 years of age. The youngest patient was 25 years old (Table 1). The age at menarche was between 10 and 15 years (mean 12.2 years) with predominantly regular menstrual cycles, while 4 patients had hormonal treatment for abnormal uterine bleeding. All the women included in the study were married and had children. Eight of them were nulliparous. The lactation periods of the women who had children ranged from eight to eighteen months.

Six of the 94 patients revealed a family history of breast cancer in their mothers, while four had family history of uterine cervix cancer, one with family history of stomach cancer, and one with colon cancer in the family. The other participants were unable to furnish pertinent family history. Of all the patients evaluated, 18 had retracted nipples, 12 presented with ulceration of the overlying skin, 6 had nipple discharge and two displayed peaud' orange appearance.

All 94 patients had fine needle aspiration cytology (FNAC), which identified 86 with ductal carcinoma, 5 with suspected malignancy, and 3 with atypical duct hyperplasia.85 of these patients had tru-cut biopsies, which verified a cancer diagnosis in 81 of them, whereas 4 patients had samples that were insufficient or unrepresentative. The remainder were taken up directly for surgery. A modified radical mastectomy with axillary clearance was performed on 87 of the patients while the remaining seven patients had undergone a simple mastectomy.

Mastectomy samples were dissected according to protocols, and lesions were detected and assessed. Three groups were created based on the size of the tumour. The majority of the patients (56.3%) had a maximal tumor diameter of 2 to 5 cm (Table 1).The most prevalent histological type (84 patients) was invasive ductal carcinoma (NOS type), with the majority (50 cases) being Grade II (Table 1). Tumor necrosis was identified on both gross examination and microscopy in 42 (44.6%) cases. Stromal lymphocytic

infiltration was observed in 22 individuals (23.4%), however no cases had stromal elastosis.

Table 1: Clinicopathological parameters of triple negative breast carcinoma(n=94)

Clinicopathological parameters	**Number of cases with percentage**
Age of the patient(years)	
25-34	4(4.2)
35-44	16(17.02)
45-54	35(37.2)
55-64	24(25.5)
65-74	12(12.7)
75-84	3(3.1)
Tumour size (cm)	
< 2	18(19.1)
2 -5	53(56.3)
>5	23(24.4)
Histological Type	
Invasive Ductal Carcinoma NOS	85(90.4)
Atypical Medullary Carcinoma	3(3.1)
Medullary	2(2.12)
Metaplastic Carcinoma	2(2.12)
Invasive lobular carcinoma	1(1.06)
Histological Grade	
Grade I	19(20.2)
Grade II	50(53.1)
Grade III	25(26.5)
Number of positive nodes	
0 - 5	58(61.7)
6-10	20(21.2)
11-15	10(10.6)
> 15	6(6.3)
Lymph vascular invasion	
Present	10(10.6)
Absent	84(89.3)
Perineural invasion	
Present	8(8.5)
Absent	86(91.4)
Ductal carcinoma *in situ*(DCIS)	
Present	20(21.2)
Absent	74(78.7)
Stromal lymphocytic infiltration	
Present	22(23.4)
Absent	72(76.5)
Tumour necrosis	
Present	42(44.6)
Absent	52(55.3)

Twenty patients (21.2%) had an in-situ component of ductal carcinoma, whereas ten patients (10.6%) exhibited lymphovascular invasion and eight patients (8.5%) had perineural invasion. In 81 cases, the surgical resection margins were clear of tumor. Margin was formed by the tumour in 12 cases and infiltration of the margins was seen in one case. Axillary node involvement studied in all patients showed involvement of all the level nodes in 36 cases, with 6 of them having more than 15 nodes.CK 5/6 expression was detected in 44 cases (46.8%) and EGFR expression was identified in 46 instances (48.9%).A total of 15 cases (15.9%) had positive results for both the markers, while 26 cases (27.5%) had negative results for both.

DISCUSSION

The study encompassed 94 patients with triple negative breast cancer. They were between the ages of 25 and 84 (mean age 50.4 years), with the largest group being over 50 (38 patients; 40.4%). This is consistent with research by Tan et al., showing that TNBC is more frequent in patients over 40 years (8) and that the commonest histological subtype is Invasive ductal carcinoma (NOS type) (90.4%; Table 1).Chen et al.,(9) reported that Invasive ductal carcinoma (NOS) constituted 97% of their patients. In line with Rakha et al., the majority of our patients (56.3%) had maximum tumour sizes between 2 and 5 cm. The high tumour sizes were ascribed to the quick pace of growth of the tumour (10). Grade II (53.1%) and Grade III (25.5%) tumours made up the majority (Table 1).This concurred with Nassar et al., who discovered that 77% of patients had high grade tumours(11).

Our analysis found 84.4% axillary lymph node positivity, which is in concurrence with Ahmed et al., reported 72.5% positivity (12). However, Bhargava et al.,(13) observed that triple negative tumors exhibit a low propensity for lymph node metastasis. Thus, whilst some have indicated no difference or even smaller lymph node involvement rates, others have documented higher prevalence of lymph node metastases. Our study revealed an in-situ component in 21.2% of cases, while Lerma et al., reported a DCIS component in 45% of cases (14).Tumor necrosis was observed in 44.6% of patients (Table 1), which was less than the 74% of patients reported by Livasy et al.,(15).Comparing the percentage of cases of stromal lymphocytic infiltration (Table 1) to the findings of Lerma et al. (49%) and Livasy et al., (56%), the percentage was lower at 23.4%.Lympho vascular invasion was identified in 10.6% whereas Billar et al.,(16) found LVI in 18%.

Immunohistochemistry highlighted the expressions of CK5/6(Fig.1) and EGFR(Fig.2). We found that a sizable fraction of TNBC exhibited at least one of the two markers. The results of Chockalingam *et al.,* and Rao et al., who discovered 74% and 67.7% of patients, respectively, with expression of CK 5/6 and/or EGFR, were consistent with this finding (17,18).

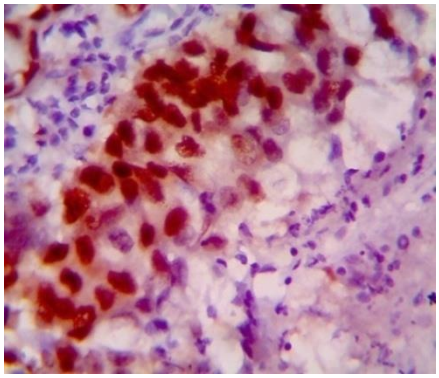

Fig.1:CK5/6 positivity in tumour cells (40X)

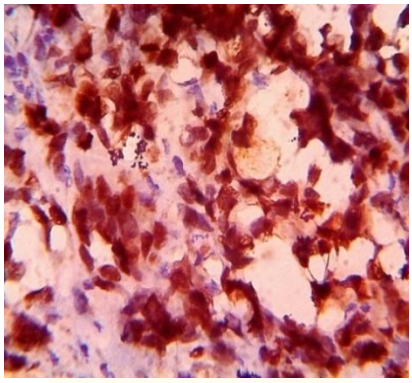

Fig.2: EGFR positivity in tumour cells(40X)

CK5/6 and clinico-pathological features

Majority of individuals with CK5/6 expression had tumors larger than 2 cm and were between the ages of 45 and 64.35 cases of invasive ductal carcinoma and all the cases with other subtypes expressed CK5/6,similar to the observations of Rao et al.,Thike et al, and Hashmi et al.,(18-20).Increased expression was seen in 23 cases of Grade 3 tumours(95.8%),which is notably higher than the observations of Thike et al.,(77%), Rao et al.,(76 %) and Hashmi et al.,(63.4%).CK5/6 positivity was high (90.9%) in cases with tumour necrosis. This observation is in concordance with that of Thike *et al.*, (98%) and Hashmi *et al.*, (96.4%;19,20). The expression of CK5/6 was found to be closely linked to the patients' age and tumour variables such as size, histological type, grade, and tumour necrosis(Tables 2 and 3).

EGFR and clinico-pathological features

In line with earlier research, most patients (59%) exhibiting EGFR expression were found to be between the ages of 45 and 64, with tumor sizes varying between 4-5 cm (Table 4).37 instances of invasive ductal carcinoma and all other subtypes exhibited EGFR, consistent with previous findings. EGFR was expressed by 91.6% (Table 5) of Grade III tumors, which is greater than in prior research. EGFR expression was high in cases of tumour necrosis (84.7%), which is consistent with Thike *et al.*, (19) and Hashmi *et al.*, (20).

Table 2: Correlation between clinico-pathological features and CK 5/6 expression

Clinico pathological features		**CK5/6**		**P value**
		Positive	**Negative**	
Age(years)	25-34	1	3	0.007
	35-44	4	12	
	45-54	13	22	
	55-64	13	11	
	65-74	10	2	
	75-84	3	0	
Tumour size(cm)	<2	1	17	0.007
	2-5	23	30	
	>5	20	23	

Table 3: Correlation between CK 5/6 expression and histopathological parameters

Histopathological parameters		**CK5/6**		**P value**
		Positive	**Negative**	
Histologic type	IDC NOS	35	50	0.026
	Atypical medullary	3	0	
	Metaplastic Carcinoma	2	0	
	Medullary	2	0	
	Invasive Lobular	1	0	
	Granular cell tumour	1	0	
Histologic grade	I	1	18	<0.0001
	II	19	31	
	III	23	1	
Tumour necrosis	Present	40	1	<0.0001
	Absent	4	49	

Table 4: Correlation between EGFR expression and clinico-pathological features

Clinico-pathological features		EGFR		P value
		Positive	Negative	
Age(years)	25-34	1	3	0.01
	35-44	5	12	
	45-54	13	22	
	55-64	14	9	
	65-74	10	2	
	75-84	3	0	
Tumour size(cm)	<2	2	16	0.042
	2-5	23	30	
	>5	16	23	

Table 5: Correlation between EGFR expression and histopathological parameters

Histopathological parameters		EGFR		P value
		Positive	Negative	
Histologic type	IDC NOS	37	46	0.268
	Atypical medullary	2	1	
	Metaplastic	2	0	
	Medullary	2	0	
	Invasive Lobular	2	1	
	Granular cell tumour	1	0	
Histologic grade	I	1	18	<0.0001
	II	23	28	
	III	22	2	
Tumour necrosis	Present	39	2	<0.0001
	Absent	7	46	

There was a substantial association between EGFR expression and patients' age and tumour variables such as size, histological grade, and tumour necrosis (Tables 4 and 5). The key histologic features of tumours with a basal-like phenotype, such as high tumour grade, high nuclear grade, increased mitotic rate and tumour necrosis were noted in the current study.

CONCLUSION

Breast carcinoma exhibiting a triple negative immunophenotype is a unique group of breast cancer, that warrants a thorough search for biological markers that might serve as possible predictors of tumour behavior and/or targets for potential therapeutic agents. Formulating an effective treatment for this subtype is quite challenging as they lack obvious drug targets like ER or HER2.In resource-constrained settings, the basal-like phenotype with aggressive clinical behavior and dismal clinical outcome can be diagnosed among triple negative breast tumors by coupling the time-honored gross and histological examinations with the application of the basal markers like CK 5/6 and EGFR.This is a cost effective and efficient measure that provides a deep insight into such an aggressive tumour and thus may be critical in designing personalized treatment strategies for patients.

CONFLICT OF INTEREST

All authors declare no conflict of interest in this work.

REFERENCES

1. Sung, H., Ferlay, J., Siegel, R.L., Laversanne, M., Soerjomataram, I., Jemal, A., *et al.*, Global cancer statistics 2020: GLOBOCAN Estimates of incidence and mortality worldwide for 36 cancers in 185 Countries. CA Cancer J Clin. 2021;71(3):209-249.
2. Mehrotra, R., Yadav, K. Breast cancer in India: Present scenario and the challenges ahead. World J Clin Oncol. 2022;13(3):209-218.
3. Chopra, R. The Indian scene. J Clin Oncol.2001;19(18 Suppl):106S-111S.
4. Munjal, K., Ambaye, A., Evans, M.F., Mitchell, J., Nandedkar, S., Cooper, K. Immunohistochemical analysis of ER, PR, Her2 and CK5/6 in infiltrative breast carcinomas in Indian patients. Asian Pac J Cancer Prev. 2009;10(5):773-778.
5. Kumar, V., Abbas, A., Aster, J. Robbins and Cotran pathologic basis of diseases. 10th ed. Amsterdam: Elsevier;2020.p.1046-1050.
6. Rakha, E., Reis-Filho, J.S. Basal-like breast carcinoma: From expression profiling to routine practice. Archives of Pathology and Laboratory Medicine.2009;133(6):860-868.
7. Elston, C.W., Ellis, I.O. Pathological prognostic factors in breast cancer. I. The value of histological grade in breast cancer: Experience from a large study with long-term follow-up. Histopathology. 2002;41(3A):151-152.
8. Tan, G.H., Taib, N.A., Choo, W.Y., Teo, S.H., Yip, C.H. Clinical characteristics of triple-negative breast cancer: experience in an Asian developing country. Asian Pac J Cancer Prev. 2009;10(3):395-398.
9. Chen, J.H., Agrawal, G., Feig, B., Baek, H.M., Carpenter, P.M., Mehta. R.S., *et al.*, Triple-negative breast cancer: MRI features in 29 patients. Ann Oncol. 2007;18(12):2042-2043.
10. Rakha, E.A., El-Sayed, M.E., Green, A.R., Lee, A.H., Robertson, J.F., Ellis, I.O. Prognostic markers in triple-negative breast cancer. Cancer. 2007;109:25-32.

11. Nassar, A., Sookhan, N., Santisteban, M., Bry-Ant, S.C., Boughey, J.C., Giorgadze, T., *et al.*, Diagnostic utility of snail in metaplastic breast carcinoma. Diagnostic Pathology.2010; 26(5):76.
12. Ahmed, M.Y., Samia, M., Mostafa, S., Mohammed, M.A., Hebat Allah, A. Clinicopathological characteristics of triple-negative breast cancer. Med. J. Cairo Univ. 2015; 83(1):797-803.
13. Bhargava, R., Striebel, J., Beriwal, S., Flickinger, J.C., Onisko, A., Ahrendt, G., *et al.*, Prevalence, morphologic features and proliferation indices of breast carcinoma molecular classes using immunohistochemical surrogate markers. Int J Clin Exp Pathol. 2009;2(5):444-455.
14. Lerma, E.,Peiro, G. Immunohistochemical heterogeneity of breast carcinomas negative for estrogen receptors, progesterone receptors and Her-2-neu (basal-like breast carcinomas). Mod Pathol.2007;20: 1200-1207.
15. Livasy, C.A., Karaca, G., Nanda, R., Tretiakova, M.S., Olopade, O.I., Moore, D.T., *et al.*, Phenotypic evaluation of the basal-like subtype of invasive breast carcinoma. Mod Pathol. 2006;19(2):264-271.
16. Billar, J.A., Duek, A.C., Stuky, C.C., Gray, R.J., Wasif, N., Nrthfelt, D.W., *et al.*, Triple-negative breast cancers: Unique clinical presentations and outcomes. Ann. Surgical Oncology. 2010;3:384-390.
17. Chockalingam, C., Rao, L., Rao, S. Clinico-pathological characteristics of triple negative and non-triple negative high grade breast carcinomas with and without basal marker (CK5/6 and EGFR) expression at a rural tertiary hospital in India. Breast Cancer (Auckl). 2012;6:21-29.
18. Rao, C., Shetty, J., Prasad, KH. Immunohistochemical profile and morphology in triple – negative breast cancers. J Clin Diagn Res. 2013;7(7):1361-1365.
19. Thike, AA., Cheok, PY., Jara-Lazaro, AR., Tan, B., Tan, P., Tan, PH. Triple negative breast cancer: clinicopathological characteristics and relationship with basal-like breast cancer. Mod Pathol. 2010;23(1):123-133.
20. Hashmi, AA., Edhi, MM., Naqvi, H., Faridi, N., Khurshid, A., Khan, M. Clinicopathologic features of triple negative breast cancers: an experience from Pakistan. Diagnostic Pathology. 2014;9(1):43.