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ORIGINAL ARTICLE

Expression of E cadherin and Ki 67: Emerging Prognostic Markers in Triple-Negative Breast Cancer

Jayaprakash Shetty[1] · Chandrika Rao[1]

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Abstract

Triple-negative breast cancer (TNBC) is an aggressive subgroup of breast cancer which lacks effective target therapy. Expression of biomarkers is one of the important deciding factors for treatment strategies. The aim of this study was to evaluate expression of E cadherin and Ki 67 in relation to clinicopathological features. This prospective observational study included 141 cases of TNBC. Immunohistochemical staining was employed to analyze two biomarkers: E cadherin and Ki 67 on formalin-fixed paraffin-embedded tumor samples obtained from patients defined as TNBC. The age of the patients ranged from 26 to 84 years. Positive lymph nodes were found in 82 (58.1%). The tumor was grade 3 in 105 (74.4%). The E cadherin receptor was positive in 83 (58.8%). The Ki Index was > 10% in 89 (63.12%). The Ki 67 expression was significantly associated with a high nuclear grade ($p = 0.000$). The significant association noted between loss of E cadherin expression and positive lymph node ($p = 0.0296$). According to the results, TNBCs are frequently associated with the younger age groups, and the majority is poorly differentiated. The majorities of these have high expression of the Ki 67 and significantly associated with the higher nuclear grade. Loss of E cadherin was significantly associated with positive lymph nodes. Hence, evaluating the expression of E cadherin and Ki 67 routinely would be helpful for evaluating prognostic implications.

Keywords E cadherin · Ki 67 · Triple-negative breast cancer · Prognostic markers

Introduction

Breast cancer is leading cancer in women across all states in urban India [1]. The crude rate and age-adjusted risk vary from 12.7–34.8 to 13.9–41 per 1,00,000 populations across several states involved in the ICMR sponsored population-based cancer registry program [2].

DNA microarray profiling of breast cancer has resulted in classification of breast cancer into five types: luminal A, luminal B, HER-2, normal breast like, and basal like [3]. These classifications are useful in that they reflect the therapeutic efficacies of hormonal therapy, molecularly targeted drug therapies using trastuzumab, and chemotherapy.

Classification of breast cancer based on DNA microarray profiling in the clinic is difficult, and today there is growing use of subclassification based on results of immunohistochemical staining for estrogen receptor, progesterone receptor, and HER-2.

TNBC represents approximately 15% of all breast cancers which lacks expression of the estrogen and progesterone receptor by immunohistochemistry (IHC) and lack overexpression and/or amplification of HER-2 obtained by IHC and/or fluorescence in situ hybridization. TNBC is currently treated with taxanes, alkylation agents, platinum agents, etc. Various issues complicate the decision of therapeutic strategy such as relatively early recurrence.

TNBC is a heterogeneous disease which includes different molecular subtypes, such as basal like and Claudin low [4]. The expression profiles of various biomarkers of TNBC are gradually being elucidated in the hope that the results would aid in therapeutic strategy decisions for TNBC. The aim of this study is to discover additional prognostic markers that can better classify TNBC and identify tumors with more aggressive behavior.

In the present study, associations between the expression of various biomarkers and the clinicopathological findings for TNBC were investigated. For this purpose, we investigated the expression of E cadherin and Ki 67 in TNBC and explored their correlations with morphological characteristics.

✉ Chandrika Rao
Chandrika_valal@yahoo.com

[1] Department of Pathology, K S Hegde Medical Academy, Deralakatte, Mangalore, India

Table 1 E cadherin and Ki 67 expression

Molecular determinants	*N* (%)
E cadherin	
Positive	83/141 (58.8%)
Negative	58/141 (41.1%)
Ki 67	
> 10%	89/141 (63.1%)
≤ 10%	34/141 (24.1%)
Negative	18/141 (12.7%)

Materials and Methods

This observational, prospective study included 141 consecutive cases of TNBC diagnosed at the Department of Pathology, K S Hegde Medical Academy, Deralakatte, Mangalore, and was based on histopathology and immunohistochemistry. Each case was reviewed with regard to clinicopathological parameters including age at diagnosis, tumor type, tumor size, tumor grade, and lymph node metastasis. Histologic grade was assessed by modified Bloom-Richardson scoring system. Histologically, confirmed invasive breast cancer cases were included. TNBC was defined based on immunohistochemistry proved ER, PR, and HER-2 negativity. Only Modified radical specimens were included. Patients who had undergone chemotherapy prior to surgery were excluded from the study.

Table 2 Tumor characteristics of patients

Variables	*N* (%)
Median age	47 years (range 26–84)
Histological type	
IDC NST	120 (85.1%)
Mixed (ILC + IDC)	2 (1.4%)
Medullary carcinoma	12 (8.5%)
Pleomorphic lobular carcinoma	5 (3.5%)
Metaplastic carcinoma	2 (1.4%)
Tumor grade	
Grade 2	36 (25.5%)
Grade 3	105 (74.4%)
Tumor size	
0–2 cm	11 (7.8%)
> 2–5 cm	105 (74.4%)
> 5 cm	25 (17.7%)
Lymph node status	
Negative	59 (41.8%)
1–3	50 (35.4%)
> 3	32 (22.6%)

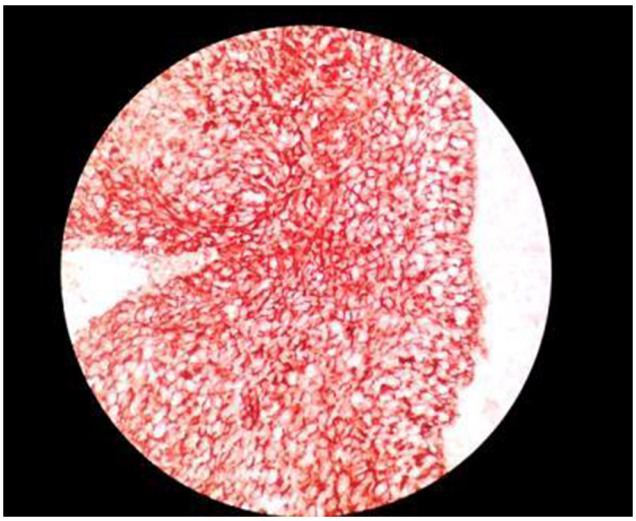

Fig. 1 Tumor showing E cadherin membrane positivity with E cadherin antibody (× 10)

Estrogen and progesterone receptor were scored negative when the proportion of positively stained cells was <1% (Allred score). Negative HER-2 expression was determined based on ASCO/CAP guidelines. Cells stained for Ki 67 were counted and expressed as a percentage. The percentage was determined by the number of Ki 67 positive cells among the total number of counted tumor cells. High expression of Ki 67 was defined as more than 10%. [5]

E cadherin expression was considered positive based on membrane positivity.

Immunohistochemistry

Estrogen receptor—Rabbit monoclonal, clone EP1,

Progesterone receptor—rabbit monoclonal, clone EP2

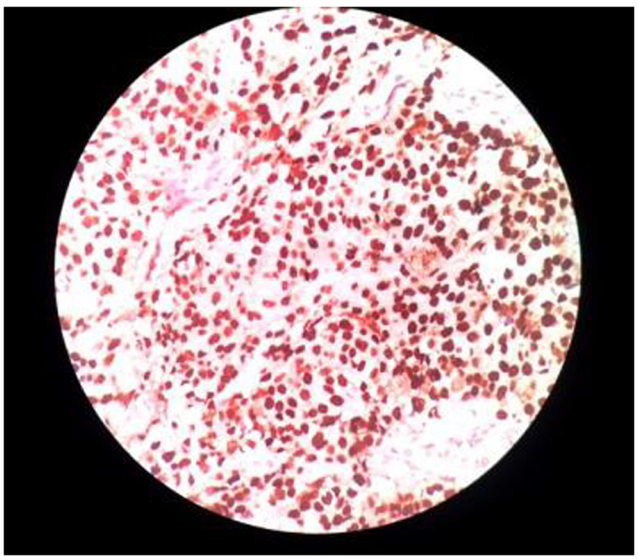

Fig. 2 Tumor showing high Ki 67 expression in triple-negative breast cancer tissue stained with Ki 67 antibody (× 10)

Table 3 Ki 67 and E cadherin correlation with tumor characteristics

Variable	Ki 67				E cadherin		
	≤ 10%	> 10%	Negative	*p* value	Positive	Negative	*p* value
Age (years)							
> 40	4	19	4		16	11	
41–50	17	35	11	0.255	38	25	0.932
> 50	13	35	3		29	22	
Histologic type							
IDC NST	26	77	1		74	46	
Pl lobular	2	2	0		1	4	
Mixed (IDC + ILC)	1	1	0		1	1	
Medullary	3	9	0		7	5	
Metaplastic	2	0	0	0.224	0	2	0.169
Grade							
2	10	15	11		24	12	
3	24	74	7	0.000	59	46	0.270
Tumor size (cm)							
≤ 2	4	5	2		6	5	
> 2–5	24	66	15		65	40	
> 5	6	18	1	0.469	12	13	0.427
LN status							
Negative	17	36	6		41	18	
Positive	17	53	10	0.463	42	40	0.029

HER-2—rabbit monoclonal, clone EP3
E cadherin—rabbit monoclonal, clone EP6
Ki67—mouse monoclonal, clone GM001
Regulatory status—IVD

Path in situ immunohistochemistry kits were used to detect expression of estrogen receptor, progesterone receptor, HER-2, E cadherin, and Ki 67.

Statistical Analysis

Data were analyzed using SPSS version 20.0. The chi-square test was conducted to analyze the clinicopathological data. $p < 0.05$ was considered statistically significant.

Results

We analyzed 141 consecutive patients of TNBC, the E cadherin and Ki 67 expression (Table 1).

Patient and tumor characteristics are summarized in Table 2.

Median age 47.0 years (range 26–84). Total 63 (44.6%) were between the age group of 41–50 years. The main histological type was ductal, no special type in 120(85.1%) patients, mixed (IDC + ILC) in 2(1.4%), medullary in 12 (8.5%), pleomorphic lobular in 5 (3.5%), and metaplastic carcinoma in 2 (1.4%). Tumor size was 0–2 cm in 11 (7.8%), > 2–5 cm in 105 (74.4%), and > 5 cm in 25 (17.7%).

Tumor grade was 2 in 36 (25.5%) and 3 in 105 (74.4%). Metastasis in 1–3 lymph nodes noted in 50 (35.4%) and > 3 lymph node involvement noted in 32 (22.6%). Total 59 (41.8%) patients had negative lymph nodes.

The E cadherin receptor was positive in 83 (58.8%). The Ki index was > 10% in 89 (63.12%) (Table 1) (Fig. 1and Fig. 2).

E cadherin showed no significant correlation with age, tumor size, tumor type, and tumor grade. A significant association was noted between loss of E cadherin expression and positive lymph node ($p = 0.0296$). Ki 67 showed significant association with tumor grade (p value 0.000) (Table 3).

Table 4 Association between E cadherin and Ki 67

	Ki 67 ≤ 10%	Ki 67 > 10%	Ki 67 negative	*p* value
E cadherin positive	20	52	11	
E cadherin negative	14	37	07	0.977

Further, no significant association noted between E cadherin expression and Ki 67 expression (p value = 0.977) (Table 4).

Discussion

Breast cancer is leading cancer in women across all states in urban India. Triple-negative breast cancer is an aggressive subset and represents a molecular subtype without specific target therapy [4].

TNBC is a heterogeneous disease and the identification of the molecular prognostic markers is necessary to allow better characterization of this subtype and for the construction of appropriate therapeutic strategies [4].

Currently, conventional chemotherapy is the main treatment modality for TNBC in the neoadjuvant or adjuvant setting. However, more than one-half of TNBCs do not respond to chemotherapy.

Recently, a new panel of biomarkers was identified to provide both prognostic and predictive information in TNBC. Among them, some of the most promising markers are E cadherin and Ki 67 expression.

E cadherin is a cell adhesion molecule. Loss of E cadherin is a fundamental event in the epithelial-mesenchymal transition, which is associated with carcinogenesis of TNBCs. Reduced E cadherin expression may also have a role in the poor prognosis in TNBCs [6].

Loss of E cadherin expression is associated with larger tumor size, higher tumor grade, and lymph node metastasis in breast cancer but reports in TNBC results remain limited and inconsistent. In the present study, there was no significant association between E cadherin expression and prognostic markers (age, tumor size, and grade).

Few studies have been reported evaluating the prognostic role of E cadherin expression, either alone or in combination with other prognostic markers. Ricciardi et al. found that lack of E cadherin expression is negatively associated with overall survival. Tang et al. found that positive lymph node status is associated with loss of E cadherin expression with a p value of 0.016. In the present study, a significant association was noted between loss of E cadherin expression and positive lymph node ($p = 0.0296$) [7].

High Ki 67 expression is associated with higher histologic grade, larger tumor size, positive lymph node status, short disease-free survival, and overall survival in breast cancer [4, 8, 9].

Currently, there is no uniform consensus on a standardized cutoff value that might be used in clinical practice, although staining levels of 10–20% have been the most common to dichotomize populations used [4, 10].

We confirmed that high levels of Ki 67 expression are significantly associated with high tumor grade; this indicates an increased proliferation of tumor cells, enhanced invasiveness, and faster growth.

Researchers found a positive correlation between the reduction in E cadherin expression and high Ki 67 expression may be associated with tumor aggressiveness, which provides insight into the role of E cadherin in TNBC tumor biology [6]. However, in the present study, there was no significant association noted between E cadherin expression and Ki 67 expression (p value = 0.977).

Conclusion

Our data suggest that TNBCs are associated with the younger age groups, and a majority are poorly differentiated with high Ki 67 expression and a significant number of lymph node positive cases show loss of E cadherin expression. Analysis of E cadherin expression and Ki 67 expression would be helpful for evaluating prognostic implications.

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