Expression of E-Cadherin in breast carcinomas and its association with other biological markers – a prospective study

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Abstract

Context E-cadherin (E-CD) is an important cell adhesion molecule in normal epithelial cells and has been shown to be an invasion tumor suppressor gene.

Materials and methods Various clinicopathological parameters like age, family history, tumor stage, histological grade, lymph node status and other biological markers were also analyzed. Present study reveals E-CD expression in 65 cases of breast cancer including 41 (63%) cases of pure infiltrating ductal carcinoma (IDC), 11 (16.9%) of pure infiltrating lobular carcinoma (ILC); another 10 (15.3%) of mixed ductal/lobular type, and remaining 3 (4.6%) miscellaneous types.

Results Negative E-CD expression was noticed more in advancing age groups ($P = 0.01$). About 59.2% cases showing negative E-CD expression had family history of breast and/or other cancers. E-CD expression was found significantly higher in cases of pure IDC (55.5%) than in pure ILC cases (18.1%) ($P = 0.04$). Eleven (68.7%) of the total 16 high-grade IDC cases, revealed negative expression. Both cases of comedo carcinoma revealed negative expression. Three (30%) out of 10 mixed cases revealed negative expression in both ductal and lobular areas, while in remaining 7 cases, positivity was seen in ductal areas only. Invasive cribriform and medullary carcinoma revealed a stronger expression, while negative staining was observed in sweat gland carcinoma. E-CD re-expression was noticed in lymph node tumor deposits. A direct association of E-CD expression with ER expression and an inverse association with that of p53 were also observed ($P = 0.001$), ($P = 0.04$).

Conclusions E-CD expression is useful, but limited, in differentiating IDCs from ILCS. Its negative expression correlates with certain poor prognostic parameters reflecting its use as a marker for invasive cancers. It re-expresses at metastatic sites.

Keywords Breast carcinomas · Immunohistochemistry · E-cadherin

Introduction

Breast cancer is one of the most frequent female malignancies across the world, as well as in India.¹ However, in India, about 60–80% of these cases present at a locally

advanced stage.² Several molecular components related to development of breast carcinomas and associated with therapeutic and prognostic value, viz. p53, RB, ER, CerbB-2; have been studied in great detail.^{3–5} Recent emphasis is on identifying markers related to tumor metastasis, which is the major cause of morbidity and

mortality in these patients'.⁶ Certain specific cell-to-cell adhesion molecules have been found to be responsible for embryonic development. One such molecule is a family of 'Cadherins'. It is a group of genetically related trans-membrane glycoproteins, involved in calcium-dependent cell-to-cell adhesion mechanism and are subclassified, based on their binding characteristics and tissue distribution like E-, P-, N-cadherins and others.^{7,8} At the structural level, E-cadherin (E-CD) has extracellular portion responsible for homophilic cellular interaction and an intracellular part that provides link to actin cytoskeleton through an association with various catenins, of which β -catenin has significant role in cell adhesion and signal transduction.^{9,10} A positive association between abnormal E-CD expression and occurrence of invasion and metastasis has been reported in cancers of stomach, breast and other organs.^{11–13} Berx et al.¹⁴ have reported that protein-truncating mutations in extracellular part of E-CD are responsible for lack of E-CD expression, thereby resulting in characteristic scattered tumor cell growth in infiltrative lobular breast cancer, which further has a propensity for dissemination. Present study was undertaken to analyze E-CD expression in various types of breast carcinomas, especially for studying its role in differentiating infiltrating lobular carcinomas (ILCs) from infiltrating ductal carcinomas (IDCs), in confused histologic settings and in investigating its importance in predicting invasive potential in different cancer types by correlating its expression in primary tumor with metastatic lymph node deposits. Its expression was also correlated with various clinical, histopathological and biological prognostic parameters.

Material and methods

A total of 65 cases of breast cancer were included for present study. Cases were categorized into different histomorphological types, viz. infiltrating duct carcinomas or IDC (41), Infiltrating lobular carcinomas (ILC) 11, mixed (10), and three miscellaneous cases. Histopathological grading was done using Nottingham's modification of Bloom-Richardson¹⁵ system in 41 pure IDCs and 10 mixed (IDC and ILC) cases. Sixteen cases had family history of breast and/or ovarian carcinoma (first and second-degree relatives). TNM staging was available in 45 (69.2%) cases.

Immunohistochemistry

Immunostaining for E-CD (Dako Corporation, Clone NCH-38 Carpinteria, CA, USA), ER (monoclonal mouse anti-Human ER α , Dako Corporation, Clone 1D5), p53 (mouse monoclonal antibody, Dako Corporation, Clone DO7) and CerbB-2/Her2-neu onco-protein (polyclonal rabbit antibody, Dako Corporation, Code A485), was

performed in all 65 cases by streptavidin-biotin method. Target antigen retrieval for E-CD immunostaining was done by immersion of sections in citrate buffer (pH 6.9), followed by microwave heating at 150°C and further, overnight exposure to primary antibody in the refrigerator at a temperature of 2–8°C.¹⁶ E-CD expression was also studied in 31 lymph nodes, showing presence of metastatic tumor deposits.

Interpretation

E-CD staining was identified at cell borders in form of granular membranous positivity (normal/positive staining). Positive controls included both intrinsic, in form of benign breast ducts and lobules, as well as external controls in the form of positive cases. Negative controls were also employed in all cases.

Immunoreactive tumor cells were semi-quantitatively estimated: grade 0 corresponded to nil staining, grade 1 to 5–10% positive cells, grade 2 to 10–50% cells and grade 3 to more than 50% cells.¹⁷ Grade 3 was interpreted as positive staining. For the other biological markers like ER, CerbB-2 and p53, >10% tumour cell nuclear positivity in case of ER, >75% membranous positivity with CerbB-2/Her2-neu and >5% nuclear positivity in tumor cells with p53 antibody were interpreted as positive staining.

Statistical methods

The significance of associations between E-CD expression with other parameters was evaluated through Chi-square (χ^2) test/Fisher's exact test (two tail). P value ≤ 0.05 was considered statistically significant (S). Odds ratio (OR) along with 95% confidence intervals (CI) was also estimated. Logistic regression analysis was attempted to evaluate the independent prognostic markers associated with E-CD expression. This was carried out with variables revealing significance in univariate analysis. Extent of agreement between E-CD expression with biological markers was evaluated by kappa (κ) value.

Results

The age of the patients, under study ranged from 24 to 74 years, with maximum number being in the age group of ≥ 50 years (Table 1). Negative E-CD expression was present in 31 (47.6%) cases. Negative E-CD expression was observed with increasing age, with 30% cases showing negative expression in age group ≤ 39 years, in contrast to 70% cases showing negative expression in the age ≥ 50 years ($P = 0.03$ and $P = 0.01$) (OR = 5.4).

Table 1. E-CD expression in different tumor age-groups ($N = 65$).

		No. of cases showing E-CD expression (grade-wise)									
		Negative				Positive					
Age (years)	No. of cases (%)	0	1	ST	%	2	3	ST	%	OR	95% CI
≤39	20 (30.7)	1	5	6	30.0	9	5	14	70.0	1.0	–
40–49	25 (38.4)	6	5	11	44.0	9	5	14	56.0	1.8	0.47–7.65
≥50	20 (30.7)	10	4	14	70.0	3	3	6	30.0	5.4	1.17–27.13
Total	65 (100)	17	14	31	47.6	21	13	34	53.8		–

ST, Subtotal cases; $\chi^2 = 6.64$, $P = 0.03$ (S); OR, Odds ratio; CI, Confidence interval; N, Total number of cases χ^2 trend = 6.31, $P = 0.01$ (S)

Table 2. E-CD expression in different types of breast carcinoma ($N = 65$)

Morphological types of tumor	No. of cases showing E-CD expression (grade-wise)				
	No. of cases (%)	Negative	%	Positive	%
Infiltrating ductal carcinoma (Pure IDC, NOS)	38(58.4)	16	44.4	22	57.8
Infiltrating ductal carcinoma (Pure IDC, Comedo type)	2	2	–	0	0
Paget's disease (Pure IDC)	1	0	0	1	–
Infiltrating lobular carcinoma (Pure ILC)	11 (16.9%)	9	81.8	2	18.1
Mixed (ILC and IDC)	10 (15.3)	3	30.0	7	70.0
Miscellaneous: Infiltrating cribriform carcinoma	3 (4.6)	1	33.3	2	66.6
	1	0	0	1	–
Sweat gland carcinoma	1	1	–	0	0
Medullary carcinoma	1	0	0	1	–
Total no. of cases	65 (100)	31	47.6	34	52.3

ST, Subtotal cases; IDC. (NOS) vs ILC, $P = 0.04$ (S)

Among 16 (59.2%) cases having family history of breast and/or other cancers, 12 were IDC cases, 2 of mixed type and 1 case each of ILC and medullary type. Negative E-CD expression was found in 7 (43.7%) cases, including 6 (54.5%) IDC cases and 1 ILC case. Both cases of mixed type along with 1 case of medullary carcinoma showed positive staining.

Negative E-CD expression in various tumor stages ranged from 38–50%, without showing any significant pattern, except for complete lack of staining in the two cases of stage 4 ($P > 0.05$).

Out of total 65 cases, 41 (63%) were of pure IDC (NOS 38 cases, 2 with comedo necrosis and 1 of Paget's disease); 11 (16.9%) of pure ILC; another 10 (15.3%) of mixed ductal and lobular type, and remaining 3 (4.6%) miscellaneous types, comprising 1 case each of infiltrating cribriform, sweat gland type and, medullary carcinoma, respectively. Out of 41 cases of pure IDC, lack of E-CD expression was seen in 18 (43.9%) cases (Table 2). Both cases of comedo IDC also revealed negative expression. Out of 11 pure ILCs, negative expression was noticed in 9 (81.8%) cases. None of the ILC cases showed 'aberrant' membranous positivity, while 2 (18.1%) showed cytoplasmic positivity in 10–50%

tumor cells. Both these cases were younger patients (<30-year-old). The differences in the negative E-CD expression between IDC and ILC cases were significant ($P = 0.04$). Out of 10 mixed types, 3 (30%) cases showed lack of E-CD expression in both ductal and lobular components. In the remaining 7 cases, positive staining was seen in ductal components, but lost in areas of lobular differentiation. Among the 3 miscellaneous types, strong E-CD expression was seen in cases of invasive cribriform and medullary type, but lost in the case of sweat gland carcinoma.

Correlation of E-CD expression with varying histologic grades in all 41 cases of pure IDC, revealed, a significant increase in negative staining with increasing grades of IDC ($P = 0.01$) (Table 3). However, among 10 mixed cases, comprising 5 (50%) cases, each of low and intermediate grade types, negative expression was noticed in 40% (2/5) cases of low-grade and in 20% (1/5) cases of intermediate grade mixed IDCs. Among 2 such low-grade cases of IDC, showing negative E-CD expression, the predominant tumor component was lobular.

Out of 65 cases, 31 (47.6%) had metastatic lymph node deposits. No significant difference in negative E-CD expression was found in primary tumor with lymph node

Table 3. E-CD expression in different tumor-grades ($N = 41$) (Pure IDCs: NOS and comedo types).

Tumor grade	No. of cases showing E-CD expression (grade-wise)			
	No. of cases (%)	Negative	%	Positive
Low-grade (I)	9 (21.9)	0	0	9
Intermediate-grade (II)	16 (41.0)	6	37.5	10
High-grade (III)	16 (41.0)	11	68.5	5
Total no. of cases	41 (100)	17	43.5	24

ST, Subtotal cases; $\chi^2 = 8.93$, $P = 0.01$ (S); N , Total number of cases; χ^2 trend = 8.90, $P = 0.003$ (S)

Table 4. E-CD expression with other biological markers ($N = 65$).

Biological marker	E-CD expression				κ value	OR	95% CI
	Expressivity (%)	Negative	Positive	Subtotal (%)			
ER	Positive	5 (17.8)	23 (82.1)	28 (43.0)	$\kappa = 0.51$	1.00	2.89–43.78
	Negative	26 (70.2)	11 (29.6)	37 (57.0)		10.87	
p53	Positive	23 (58.9)	16 (41.0)	39 (60.0)	$\kappa = 0.35$	3.23	1.01–10.63
	Negative	8 (30.7)	18 (69.2)	26 (40.0)		1.00	
CerbB-2	Positive	19 (46.3)	22 (53.6)	41 (63.0)	$\kappa = 0.98$	–	–
	Negative	12 (50.0)	12 (50.0)	24 (37.0)			

OR, Odds ratio; CI, Confidence interval; N , Total number of cases

metastasis (48.3%), compared to those without lymph node deposits (47.0%) ($P > 0.05$, NS). E-CD expression was also studied in lymph node deposits and compared with expression in primary tumors. Lymph node tumor deposits in all 31 (100%) cases revealed positive staining for E-CD. Out of these, in 11 (35.4%) cases, primary tumor showed negative E-CD staining, while lymph node deposits showed ‘aberrant’ positive staining. In 16 (51.6%) cases, a higher grade of E-CD staining was noted in lymph node tumor deposits, as compared to the staining in primary tumor. Positive expression was also found in lymph node vascular/lymphatic tumor emboli.

A correlation of E-CD expression was done with different biological parameters having prognostic implications, viz. ER, p53 and CerbB-2. (Table 4). Among these, ER positivity was seen in 28 (43%) cases, out of which 23 (82.1%) had positive E-CD expression. A direct association between E-CD expression and that of ER was noticed in 49 (75.3%) cases ($P = 0.001$, $\kappa = 0.51$). Expression of p53 antibody was observed in 39 (60%) cases. Forty-one (63%) cases showed a significant inverse p53 expression with E-CD staining ($P = 0.04$, $\kappa = 0.35$). CerbB-2 positivity was observed in 41 (63%) cases, with 34 (52.3%) cases showing a direct association. However, it was not found to be statistically significant ($P = 0.98$).

There was no significant difference in ER, p53 and CerbB-2 positivity in cases showing positive E-CD

expression, both in primary tumor and lymph node deposits, as compared to those showing negative E-CD expression in primary tumor and positive expression in lymph node deposits. Since all three variables, viz. age, ER and p53 expression revealed significant association with that of E-CD; further multiple logistic regression analysis carried out, revealed independent ER and p53 expression to that of E-CD.

Discussion

The most significant factor associated with breast cancer morbidity is metastasis⁶. Newer markers have been established in context of tumor dissemination. The cadherin-mediated cell adhesion system is known to act as an invasion suppressor system¹⁸ in cancer cells. Damsky and colleagues¹⁹ first identified human E-CD as cell-CAM 120/80 using polyclonal antibody. In epithelial cells, this transmembrane molecule is considered as one of the key molecules for the formation of the intercellular junctional complex and for establishment of cell polarization.²⁰ There is significant experimental evidence validating its role as a potent invasion suppressor and tumor suppressor gene.^{21–22} Several authors^{11–13} have investigated role of E-CD catenin complex in dissemination of the disease in-patients with various malignancies. Except for differential expression in lobular versus ductal carci-

nomas, there have been fewer studies reflecting different facets of E-CD expression, including its association with various prognostic indicators.

In the present study, we explored the various aspects of E-CD expression in different types of breast carcinomas and correlated the expression with various clinico-pathologic parameters and certain established biological prognostic markers like steroid hormone receptor protein (ER), oncoprotein *CerbB-2* and tumor suppressor protein *p53*.

An increase in number of cases, revealing negative expression was noticed with advancing age groups. Cases over 50 years of age revealed significant negative expression (70% cases), as compared to only 30% cases with negative expression with early onset cancer patients', i.e. ≤ 39 years of age ($P = 0.03$). Further, a 5 times increased risk of negative E-CD expression was observed in patients with age ≥ 50 years. This correlates with the poor prognosis of breast cancer in older patients.²³ Although some authors²⁴ have reported young age to be adverse prognostic factor, in the present study, younger patients showed more of positive E-CD expression. This is due to the fact that older patients generally present at a locally advanced stage, in contrast to younger patients presenting at a relatively earlier stage.

Another important observation included 59.2% cases showing negative E-CD expression with family history of breast, ovarian and/or other cancers. Lack of E-CD expression in one case of familial diffuse type of gastric carcinoma has been reported by Keller et al.²⁵ to be genetically correlating with a case of diffuse ILC in the patients' mother, as a result of a germline mutation in *CDH 1* gene. In our study, most of the familial cases had breast or ovarian cancers in their relatives. Out of 16 such cases, 1 was of pure ILC and 2 cases of mixed ILC and IDC; with loss of E-CD expression in the former as well as in areas of lobular differentiation of the latter 2 mixed cases. Analysis of gene mutation in these cases would be worthwhile in identifying the underlying cause.

As regards tumor stage association, the highlight included negative expression in both cases of stage 4 carcinoma. This seems to be in concordance with the fact that E-CD alterations occur in advanced tumor stages, thereby rendering it as a tumor invasion suppressor gene, as postulated by Frozen et al.²⁶. Otherwise, no significant association of E-CD expression was observed with tumor stage in the present study ($P > 0.04$).

Among the various histomorphological types, a significant increase in negative expression was observed in ILCs, in contrast to IDC cases, as reported by various authors.^{27–29} This property of reduced E-CD expression in ILCs has been found to be useful in strengthening a diagnosis of lobular carcinoma. In fact, the molecular 'signature' of lobular carcinoma in breast is a truncating mutation of *E-CD* gene, leading to loss of expression of

this protein and accounting for characteristic 'single cell' growth pattern of this tumor type, thereby facilitating permeation through tissue planes. However, somatic mutations in *E-CD* gene have been found in only 56% ILCs (14). Cytoplasmic positivity, as noticed in two cases of ILC in present study, has also been reported by other workers^{30,31}. This different pattern of E-CD expression in invasive ductal and lobular carcinomas suggests that this protein molecule plays different role in the two morphologic types. In cases of ductal carcinomas with lobular differentiation, we observed a mixed pattern of staining. Areas of lobular differentiation showed reduced to nil expression, in contrast to membranous positivity in areas of ductal differentiation. In the present study, out of the total 38 pure IDC (NOS) cases, 16 (42.1%) revealed negative expression. Among the various IDC types, negative expression was characteristically observed in both the cases of IDC with comedo necrosis. Salahshor et al.³² reported a germline E-CD alteration (Aa592Thr) in cases of IDCs with comedo necrosis, which was considered to be responsible for phenotypic divergence of this variant from other IDC types, thereby forming an underlying reason for its negative expression in the present study. This further substantiates aggressive behavior of this tumor type. Out of the remaining 3 miscellaneous cases, strong positivity in one case each of invasive cribriform and medullary carcinomas seems to be correlating with these good prognostic subtypes, while nil expression in sweat gland type of carcinoma further reflected, its already reported, poor prognosis. There have been recent case reports like absence of E-CD in primary small cell carcinoma of breast³³. However, individual prognosis with breast cancer is mainly assessed by grading rather than by histological type.³⁴

Among the IDC cases with varying grades, negative expression was significantly found in high-grade IDCs (68.5%), in contrast to low and intermediate grade cancers ($P = 0.01$). Gamalo et al.³⁵ also reported similar inverse association between differentiation grade of breast carcinomas and E-CD expression. Retention of E-CD expression in 31% high-grade IDC cases, with 2 cases showing strong positivity, suggests other mechanisms operating in tumor progression, apart from loss of E-CD expression.³⁶

Although E-CD has been reported as an invasion suppressor gene, in the present study, we did not find any association of E-CD expression with lymph node status^{21,35}. An interesting finding in our study included 'aberrant' positive staining in lymph node tumor deposits in all cases (100%), irrespective of its expression in primary tumor. Thirty-five percent cases showed positive E-CD staining in the deposits. Similar observations were reported in distant metastatic tumor deposits by Kowalski et al.³⁰ There is growing evidence that carcinoma cells, which loose E-CD expression, may re-express it once

they reach distant sites. Underlying reasons for this re-expression might be related to the fact that tumor cells require to gain certain molecules that would enable them to adhere to different stromal components and endothelial cells at foreign sites, which they loose at the time of being uprooted from primary site, where the expression gets lost. It has been suggested that translational regulation and post-translational events are probable mechanisms for its re-expression.³⁷ In the present study, a stronger expression was noticed in tumor emboli in the interspersed vessels and lymphatics (in 8 cases), compared to primary tumor. Re-expression in the circulating tumor cells probably enables them to survive as emboli.³⁸

Among the other immunohistochemical markers studied, ER expression significantly correlated with E-CD positivity. This direct association was found to be 51% after accounting for chance agreement. While, Lipponen et al.³⁹ demonstrated a similar direct association of E-CD expression with ER protein expression, other workers^{30,40} did not observe any significant association between the two. It has been postulated that reversible down regulation of E-CD may occur by hypermethylation of the promoter region of E-CD expression.⁴¹ Forced expression of E-CD in tumor cell lines has been shown to result in reversion from an invasive to benign tumor cell phenotype.⁴² Besides, the reversibility of E-CD down regulation has been demonstrated by the observation that the invasion suppressor activity of E-CD can be restored by

treating the tumor cells with antiestrogen tamoxifen, thereby strengthening the present results of direct association between E-CD and ER expression.⁴³ Besides, a significant inverse association of E-CD expression with p53 staining was also noticed ($\kappa = 0.35$). Bukholm et al.⁴⁴ noticed a strong down-regulation in E-CD protein expression and alterations of p23 protein and concluded that p53 protein might play a role in E-CD expression. No significant association was seen between CerbB-2 and E-CD expression ($P > 0.05$). To sum up, E-CD expression has significant role in differentiating IDCs from ILCS, with limitations, since high grade IDCs reveal negative E-CD expression like ILCs. Different pattern of E-CD expression in these two phenotypes suggests involvement of different mechanisms for its negative expression and tumor progression. Gene mutation analysis in cases of negative E-CD expression in familial cases and different morphologic types of breast cancer may contribute towards understanding role of E-CD in the various cancer types. Elucidating the mechanisms underlying re-expression of E-CD at distant tumor sites, as a result of translational regulation and post-translational events, would be an asset in identifying such markers related to tumor invasion. This would further be an asset for developing significant intervention strategies and new therapeutic modalities for combating highly invasive cancers, which are of major concern to the Indian oncosurgeons and pathologists, at their weakest.

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