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Histopathological and Immunohistochemical Study of E-cadherin in Breast Neoplasia

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Immunohistochemical staining was undertaken for E-cadherin (EC), estrogen and progesterone receptors; on tissue sections of normal breast, proliferative breast lesions and malignant breast lesions. The EC positive expression manifested itself as sharp membranous staining and was assessed semi-quantitative into four categories: 0; 1+ were considered negative immunoreactivity, while 2+ and 3+ were scored as positive immunoreactivity. Although 95% of non malignant proliferative breast lesions showed positive EC immunoreactivity and reduced or lost EC expression in all Pre-invasive cases. None of invasive lobular cases express EC, in contrast to 30% of invasive duct carcinoma cases, showed reduced or lost EC expression in high histological grades. Correlation between the EC staining intensity and IDC, ILC and TC groups, statistical analysis was highly significant only for TC ($p<0.001$). There was a significant relationship between E-cadherin expression and different breast neoplastic histological types. The molecular signature of breast lobular carcinomas is the loss of E-cadherin protein expression as evidenced by immunohistochemistry, whereas ductal carcinomas are typically E-cadherin positive. Present study suggests that E-cadherin may be involved in the pathogenesis of this form of breast cancer.

Key words: Breast, proliferative, lobular, ductal, carcinoma, E-cadherin

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INTRODUCTION

The three most commonly diagnosed cancers among women in 2006 will be cancers of the breast, lung and bronchus and colon and rectum, accounting for about 54% of estimated cancer cases in women (Jamal *et al*., 2006). Breast cancer alone is expected to account for 31% of all new cancer cases among women. Human breast carcinomas most frequently evolve from the epithelial lining of the terminal mammary ducts that may progressively become invasive and ultimately metastatic (Anonymous, 2002; O`Shaughnessy, 2006). The transformation of normal mammary epithelial cells into a carcinoma and the subsequent progression to invasion and metastasis involve the accumulation of numerous genetic hits, including the activation or amplification of dominant oncogenes and the deletion or inactivating mutation of key tumor suppressor genes (Goldstein, 2002; Graff *et al*., 2000). Absent cell-to-cell adhesion seems to be a necessary property of carcinoma cells to facilitate permeation through tissue planes and produce characteristic lobular carcinoma-type (Handschuh *et al*., 1999; Rashid *et al*., 2001). An observation of interest was that the expression of E-cadherin requires further evaluation for confirmation of a common regulatory pathway that could be activated in the early onset of nodal metastasis (Pinder *et al*., 1998; Vos *et al*., 1997). E-cadherin expression is diverse and differences in patient characteristics may produce variability in expression. E-cadherin is a transmembrane glycoprotein that mediates epithelial cell-to-cell adhesion (Acs *et al*., 2001). E-cadherin (EC) is a calcium-regulated adhesion molecule expressed in most normal epithelial tissues Whereas some studies have indicated that down regulation of e-cadherin, associated with loss of cellular adhesiveness, was correlative with poor prognosis and metastasis, other studies have failed to confirm this (Charpin *et al*., 1998; Guriec *et al*., 1996; Hunt *et al*., 1997). The present study examine the relationship between E-cadherin and other prognostic markers in breast cancer. The ER and the E-cad gene have been implicated frequently in the initiation and/or progression of human breast cancer (Bracke *et al*., 1996; Kanai *et al*., 1994; Nass *et al*., 2000). Loss of expression of either gene has been associated with poorly differentiated tumors and poorer prognosis (Larue *et al*., 1994; Berx *et al*., 1995a, b; Heimann *et al*., 2000; Siitonen *et al*., 1996). Furthermore, several studies have reported an association between E-cad and ER expression in breast tumors (De Leeuw *et al*., 1997a, b) Because loss of E-cadherin expression results in disruption of cellular clusters, it has been postulated that E-cadherin functions as a tumor suppressor protein (Gumbiner, 2000; Rubin *et al*., 2001). The role of E-cadherin in highly aggressive form of breast cancer, is largely unknown (Pierceal *et al*., 1995; Umbas *et al*., 1994; Umbas *et al*., 1997). Adhesion molecules, particularly cadherins play a pivotal role in cancer invasion and metastasis (Gumbiner, 2000; Rubin *et al*., 2001). Because the therapeutic management of tumors with and without nodal metastasis differs considerably (Heimann *et al*., 2000). Tumor cells strongly express E-cadherin, thereby providing an important exception to the positive association between E-cadherin loss and poor prognosis in breast cancer (Anonymous, 2002; O`Shaughnessy, 2006). The objective of this study was to assess whether E-cadherin expression contributes to the development and progression of different breast carcinomas and to investigate any differences in the expression of E-cadherin immunohistochemically in different cases of breast carcinomas. Histopathological study was, on formalin-fixed, paraffin-embedded tissue sections and immunostained for E-cadherin, estrogen and progesterone receptors (ER and PR, respectively). We have immunohistochemically investigated E-cadherin (E-CD) expression in a series of relationships between membrane E-cadherin reactivity of invasive carcinoma, a dyshesive growth pattern and lobular carcinoma-type Infiltrating Ductal Carcinomas (IDC) in an attempt to assess the biological and prognostic relevance of E-CD in patients harboring breast carcinomas. We evaluated the EC expression as an aid to subclassification of invasive breast carcinoma. And correlated with various clinical and pathological prognostic factors: the histologic type, grade status, tumor size, hormone receptor status (ER and PR) Immunoreactivity for estrogen receptor, progesterone receptor (in >10% of lesional cells).

MATERIALS AND METHODS

Retrieved 50 different breast lesions and breast carcinomas cases from the surgical pathology files of Kasr EL Aini Hospital in Cairo University in the period 2001 to 2002. The H and E-stained slides were reviewed to grade and subclassify the tumors based on established criteria without knowledge of immunohistochemical results. Primary tumour size was reported based on TNM system. Five micron thick sections were obtained from 10% formalin fixed tissue samples paraffin-embedded tissue samples for routine heamatoxylin and eosin stain. Histological grading after final histologic review using the Elston/Nottingham modification of Bloom-Richardson system according to Bane *et al*. (2005).

Cases were selected for study, including non malignant breast lesions 3 cases of duct papilloma, 5 cases of fibroadenoma, 3 cases of lobular hyperplasia. and malignant breast lesions including 5 cases of Duct Carcinoma *in situ* (DCIS), 4 cases of Lobular Carcinoma Insitu (LCIS), 18 cases of Invasive Duct Carcinoma (IDC), 8 cases of Invasive Lobular Carcinoma (conventional ILC), 4 cases of Tubular Carcinoma (TC). Data on tumor size, axillary lymph node status (axillary lymph nodes from radical mastectomy specimens), stage of disease, were abstracted from the pathology reports.

Immunohistochemical analysis was performed using a monoclonal antibody (clone 4A 27, Zymed, South San Francisco, CA) to EC on formalin-fixed, paraffin-embedded specimens were cut and mounted onto positive charged glass slides. Samples were deparaffinized and rehydrated before incubation for 5 min with 5% hydrogen peroxide to inhibit endogenous peroxidase. The tissue sections were treated using heat induced antigen retrieval technique. After treatment with blocking serum (to reduce the nonspecific binding of conjugated second antibody), the samples were incubated for 60 min with monoclonal antibody anti-E-Cadherin second generation prediluted antibody. Then rinsed well with Phosphate Buffer Saline (PBS). Two drops of biotinylated secondary product was put on the sections and incubated for 10 min then rinsed with PBS. Two drops of enhanced horseradish peroxidase conjugated streptavidine were added to each section, incubated for 10 min then rinsed well with PBS. The presence of peroxidase was revealed by addition of diaminobenzidine (DAB) chromogen that creates an intense brown deposit around the antigen/antibody/ enzyme complex. Finally sections were counter stained with Mayer's hematoxylin and mounted. Immunostains were evaluated independently and any differences in interpretation were resolved by simultaneous viewing. Evaluation of immunostaining and scoring was performed by light microscopy. Positive expression of the E-cadherins manifested itself as sharp membranous staining and with cytoplasmic staining in a few lesions and was statistically significant (Bukholm *et al*., 2000). Only the membrane staining intensity and pattern was evaluated on a scale of 0 to 3, scores of 0 and 1+ were considered negative immunoreactivity of 2+ and 3+ was scored as positive. Cytoplasmic staining was rare, considered nonspecific and not included in assessment. The presence of EC staining in epithelial cells of normal ducts and acini served as an internal positive control in every case (Bukholm *et al*., 2000). Negative controls in which the primary antibody was replaced with tris-buffered saline, were included in every run. All sections were first screened to disclose the areas with well preserved tissue architecture and cell morphology for scoring of immunoreactivity. Necrotic areas were discarded in the analysis count mitotic figures at periphery of tumor in most mitotically active area; count 10 high power fields in the same area, but necessarily contiguous; select fields with as much tumor as possible; avoid poorly preserved areas.

The immunostain scores were correlated with the histologic type, grade status, tumor size, hormone receptor status (ER and PR) Immunoreactivity for estrogen receptor, progesterone receptor (in >10% of lesional cells). The association between EC and tumor type was assessed by using the chi square test. Associations with ER and PR were assessed. A 2-sided p-value less than 0.05 was considered statistically significant.

RESULTS

Histological study and tumor subtypes, tumor grades along with EC immunoreactivity are summarized in Table 1.

Histologic types and E-cadherin: Correlation between E-cadherin intensity and histological grades of IDC cases studied in Table 2.

IDC and ductal special types: Positive EC expression was seen in all but 6(33.3%) cases of IDC (Fig. 1, 2 and 4; Table 1 and 2) and all 4 ductal special types (Tubular Carcinoma TC) (100%). EC expression was present in 100% of tumor cells in all positive cases and the staining

Table 1: Histologic tumor subtypes and tumor grade along with EC immunoreactivity

Histologic type/No. of cases	Grade I	Grade II	Grade III	Positive E-cadherin 3+	Positive E-cadherin 2+	Negative E-cadherin 1+	Negative E-cadherin 0
Duct papilloma (3)	-	-	-	3	-	-	-
Fibroadenoma (5)	-	-	-	5	-	-	-
Lobular hyperplasia (3)	-	-	-	-	3	-	-
Ductal carcinoma *in situ* (5)	-	-	-	3	2	-	-
Lobular carcinoma *in situ* (4)	-	-	-	-	-	-	4
Invasive duct carcinoma (18)	3	11	4	3	5	4	6
Invasive lobular carcinoma (8)	7	1	-	-	-	-	8
TC (4)	-	-	-	-	4	-	-

All invasive carcinomas were graded using The Elston/Nottingham modification of Bloom-Richardson grading system

Table 2: Correlation between E-cadherin intensity and histological grades of IDC cases

Histological grades	0 EC intensity	+1 EC intensity	+2 EC intensity	+3 EC intensity	Total cases (%)
Grade I	-	-	2	1	3 (16.6)
Grade II	3	3	3	2	11(61.1)
Grade III	3	1	-	-	4 (22.2)
Total and %	6 (33.3%)	4 (22.2%)	5 (27.7%)	3 (16.6%)	18 (100.0)

Table 3: Analysis of prognostic tumor parameters with loss of E-cadherin expression in all carcinomas (data are given as percentage)

Variable	Cases (%)	EC + (%)	EC - (%)	p-value
Size				0.317
T_1	56.5	82.4	17.6	
T_2	33.9	76	24	
T_3	4.8	62	38	
T_4	4.8	92	6	
Grade				<0.001
I	22.1	56	44	
II	41.7	79.1	20.9	
III	36.2	94	6	
Estrogen receptor				<0.001
Positive	75.4	74	26	
Negative	24.6	96	4	
Progesterone receptor				0.127
Positive	67.8	77	23	
Negative	32.2	84	16	

p-value less than 0.05 was considered statistically significant

was 2+ in 5 cases and 3+ in only 3 cases (Fig. 1, 3 and 4; Table 3 and 4). The all 4 cases of tubular carcinoma showed 3+ EC positive immunoreactivity (Fig. 4). Associated Ductal Carcinoma *in situ* (DCIS) was positive with 3+ EC immunoreactivity (Fig. 2).

Invasive lobular carcinoma: Of 8 ILCs with the classic histologic pattern, (100%) showed complete loss of EC; 2 histologically typical ILC (25%) showed faint incomplete membrane staining in less than 10% of tumor cells. Seven of these EC-negative cases were well-differentiated (nuclear grade I). Six cases IL showed loss of EC membrane staining in invasive and corresponding *in situ* components (Fig. 3 and 4; Table 1 and 2).

Statistical analysis

E-cadherin expression and tumor subtype: All nonmalignant proliferative breast lesions included in this study showed strong (3+) positive EC immunoreactivity and all cases of Duct Papilloma and fibroadenoma.

All cases of Lobular Hyperplasia showed weak (2+) positive EC immunoreactivity (Fig. 2 and Table 1). Comparison of EC staining in IDC, ILC and Tubular carcinoma (Table 1, Fig. 1, 3 and 4) TC revealed a highly statistically significant difference between the groups (p<001). Overall, negative staining of EC in ILC was specific for the diagnosis of ILC (Fig. 3).

E-cadherin expression versus tumor parameters: All Invasive Carcinomas EC expression and tumor

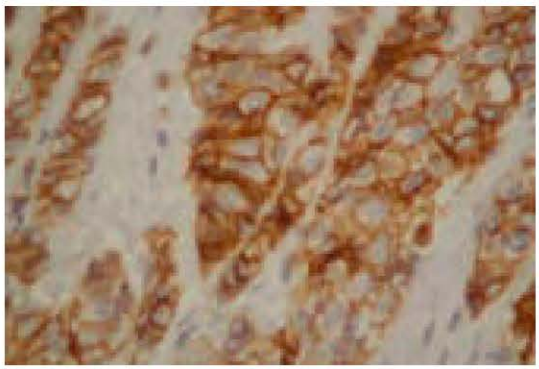

Fig. 1: Invasive Ductal Carcinoma (IDC) grade I, with strong 3 + positive immunoreactivity to E-cadherin (x200)

characteristics did not reveal statistically significant associations with loss of EC expression However, loss of EC was associated significantly with tumor grade (Table 3; Fig. 1 and 4).

All carcinomas excluding ILC grade, size and hormonal status. Complete loss of EC was seen in 6 cases of IDC and special types to be of prognostic or predictive value (Table 3). Although only (44%) of IDC expressed E-CD in >5% of tumor cells (EC-positive carcinomas), of higher histological grade (p = 0.001). In addition, E CD-positive tumors were negative for estrogen (p = 0.001) and progesterone receptors (p = 0.001) and showed reduced E-cadherin expression (p = 0.276) more frequently than E-CD-negative tumors.

DISCUSSION

In this study normal ductal and acinar epithelium, expressed EC strongly as was also demonstrated by Bukholm *et al.* (1998 and 2000). All nonmalignant proliferative breast lesions included in this study showed strong (3+) positive EC immunoreactivity as well as all cases of Duct Papilloma and fibroadenoma. All cases of lobular hyperplasia showed weak (2+) positive EC immunoreactivity (Table 1, Fig. 1 and 2). According to Bukholm *et al.* (2000) demonstrated a similar EC expression in the normal ducts and lobular epithelium and also in ductal hyperplasia, but reported marked loss of EC in Atypical lobular Hyperplasia.

In the present study all cases of LCIS showed total loss of EC expression and DCIS showed either weak or strong EC expression (40 and 60%, respectively) (Table 1, Fig. 2 and 3). Similar results have been recorded by Bukholm *et al.* (2000), where total loss of EC expression was found in all cases of LCIS cases, whereas, DCIS cases almost exhibited EC expression. Loss of EC expression

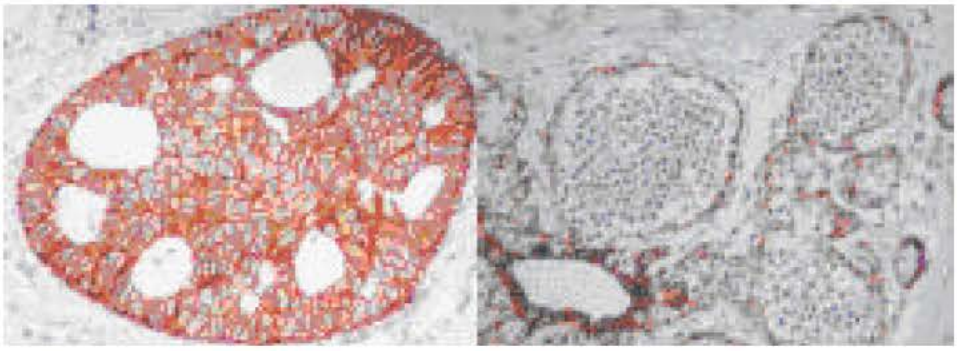

Fig. 2: Duct carcinoma *in situ* (DCIS) with strong 3+ positive immunoreactivity to E-cadherin (to the left), versus the negative staining in case of lobular carcinoma *in situ* (LCIS) to the (right); with the intact peripheral myoepithelium preserved and served as positive internal control (x200)

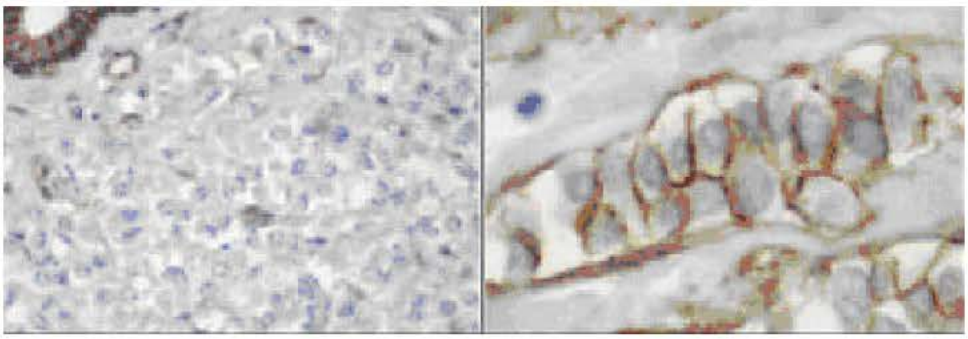

Fig. 3: E-cadherin immunostains can aid in the distinction of lobular carcinoma, EC doesn`t show significant staining of the membrane (to the left) in contrast to ductal carcinoma grade I (to the right). Invasive duct carcinoma grade I with strong 3+ positive immunoreactivity to E-cadherin. A benign duct serves as a positive internal control (x400)

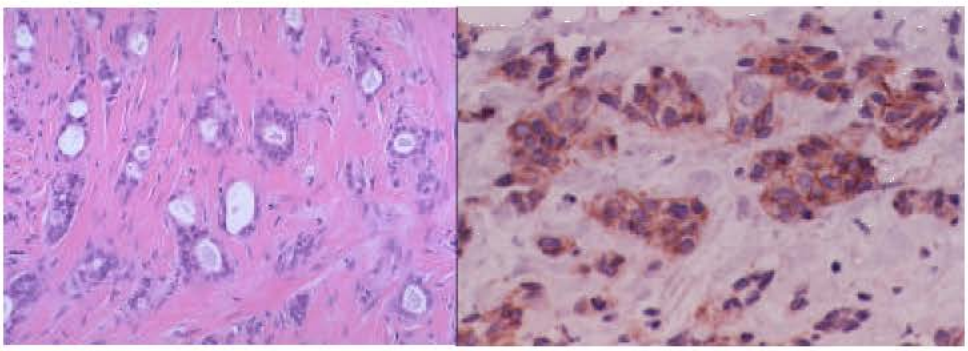

Fig. 4: Tubular carcinoma showing irregular tubular glands arranged haphazardously in a fibrotic stroma without a lobular pattern. Coexisting cribriform ductal carcinoma *in situ* is seen in the right, H and E (x100) to the left. Tubular carcinoma with strong 3+ positive E-cadherin immunoreactivity (x200) to the right

was noticed by Gumbiner (2000). Furthermore, Acs *et al.* (2001) found no expression of EC not only in ILC and LCIS adjacent to ILC but also in LCIS without an invasive component indicating a role for EC mutation even before tumour invasion. Whereas EC expression was present or reduced in DCIS, they concluded that EC is a classical tumour-suppressor gene and a very early target in lobular carcinogenesis (Pinder *et al.*, 1998; Wahed *et al.*, 2002).

Umbas *et al.* (1994 and 1997) used the term *Lobular carcinoma in situ* for a special type of noninvasive carcinoma of breast associated with a monotonous intralobular proliferation of cells.

The concurrent invasive carcinoma with absence of tubule formation and single-file growth pattern was established as ILC. (Gumbiner, 2000; Sarrio *et al.*, 2003; Wahed *et al.*, 2002). The distinctive histologic features of this special type of breast carcinoma described by Jones and Laird (1999) and Graff *et al.* (2000) paved the way for identification of this tumor by pathologists when the classic features are present. Identification of variants of ILC has added new dilemmas to the existing problem of distinguishing IDC of no special type with cord-like or trabecular patterns from ILC and its variants (Singletary *et al.*, 2002; Woodward *et al.*, 2003).

Selective EC loss, now well recognized, validates ILC as a distinct entity and explains its histologic appearance, (Jones and Laird, 1999; Graff *et al.*, 2000) and distinctive growth patterns in metastases (Singletary *et al.*, 2002; Woodward *et al.*, 2003).

Although EC is emerging as an excellent marker to type breast carcinomas (Jones and Laird, 1999; Graff *et al.*, 2000) the conflicting reports of EC loss as predictor of increased invasiveness, metastatic potential and poor survival (Jones and Laird, 1999; Graff *et al.*, 2000) raise questions about its reliability for typing. Loss of EC alone cannot be a predictor of metastatic potential and negative outcome as EC is lost even in the preinvasive stages of LCIS and atypical lobular hyperplasia (Jones and Laird, 1999; Graff *et al.*, 2000; Singletary *et al.*, 2002; Woodward *et al.*, 2003).

Furthermore, ILC is a slow-growing tumor that has been shown to have better survival than ductal no special type (Singletary *et al.*, 2002; Woodward *et al.*, 2003).

As demonstrated in this study and in previous studies by De Leeuw *et al.* (1997); Acs *et al.* (2001) and Wahed *et al.* (2002) that EC can help in the diagnosis of ILC. In this study, complete EC loss is reported in 100% of ILCs, as reported also by Singletary *et al.* (2002) and Woodward *et al.* (2003) showing good membrane positivity in all cases of IDC, including the special type Tubular Carcinoma (Fig. 1, 2 and 4, Table 1, 2 and 3). Most of the ductal invasive and in situ carcinomas cases showed strong EC-positive (3+) immunoreactivity (Fig. 1, 2 and 4). The exception was in the 4 cases of TC that showed 2+ staining in the tubules only (Fig. 4) and a very few high-grade cellular IDCs with apparent reduced expression of EC (Fig. 1, 2 and 3).

Berx *et al.* (1995) and Acs *et al.* (2001) observed variation in EC intensity in IDC, diagnostic difficulty occurs in some cases because IDC may show a dispersed growth pattern, including infiltration around benign ducts in a targetoid manner similar to ILC (Sarrio *et al.*, 2003; Woodward *et al.*, 2003).

Most studies have observed retained EC expression in almost all IDCs but reduced expression mainly associated with poor differentiation and high tumor grade (Sarrio *et al.*, 2003; Woodward *et al.*, 2003). Various studies have observed a correlation between reduced EC expression and lymph node status (Charpin *et al.*, 1998; Goldstein, 2002) and ER and PR status (Kleer *et al.*, 2001; Nass *et al.*, 2000; Rashid *et al.*, 2001; Rubin *et al.*, 2001; Seidman *et al.*, 2001) Others have found no relationship to nodal or receptor status.

Present findings were similar to Acs *et al.* (2001) and Goldstein *et al.* (2002), proved no correlation between EC expression with tumor size, grade, tubule formation, nuclear pleomorphism, mitotic activity, ER and PR status in invasive carcinomas.

Absent cell-to-cell adhesion seems to be a necessary property of carcinoma cells to facilitate permeation through tissue planes and produce characteristic lobular carcinoma-type Bukholm *et al.* (2000). An observation of interest was that the expression of E-cadherin requires further evaluation for confirmation of a common regulatory pathway that could be activated in the early onset of nodal metastasis. Loss of EC is a sensitive and relatively specific marker to confirm a diagnosis of ILC and its variants. EC positivity clearly favors ductal differentiation in ambiguous cases. Partial loss of EC in a minority of poorly differentiated IDCs is not of diagnostic significance.

CONCLUSION

All nonmalignant proliferative breast lesions included in this study showed strong (3+) positive EC immunoreactivity. There was reduced or lost (EC) expression in all Pre-invasive breast carcinomas cases.

Invasive Lobular Carcinoma (ILC) and Invasive Ductal Carcinoma (IDC) cases showed striking difference in their (EC) expression. Moderate to strong membrane expression found in all invasive (100%) and *in situ* Ductal Carcinomas.

Loss of EC alone cannot be a predictor of metastatic potential and negative outcome, as EC is lost even in the preinvasive stages of LCIS and atypical lobular hyperplasia.

All invasive (100%) and *in situ* lobular carcinomas showed complete loss of E-cadherin expression. Lobular and not ductal *in situ* neoplasia displays loss of E-cadherin expression has greatly facilitated the categorization of a large proportion of morphologically ambiguous intraepithelial neoplasias into ductal or lobular types.

These results suggest that although E-cadherin expression may be involved in the progression of IDCs, its value as an independent prognostic factor remains to be established. The level of decreased E-cadherin expression at which a dyshesive growth pattern emerges in primary breast carcinomas may be less than the level associated with lobular carcinoma-type.

Tubular Carcinoma revealed a highly statistically significant difference between the groups ($p<001$). Overall, negative staining of EC in ILC was specific for the diagnosis of ILC.

Loss of EC is a sensitive and relatively specific marker to confirm a diagnosis of ILC and its variants. EC positivity clearly favors ductal differentiation in ambiguous cases. Partial loss of EC in a minority of poorly differentiated IDCs is not of diagnostic significance. Present study suggests that E-cadherin may be involved in the pathogenesis of this form of breast cancer We considered the inherent loss of EC in all lobular breast carcinoma and the expression of other prognostic tumor variables that previous studies have not considered. There was a significant relationship between E-cadherin expression and different breast lesions and carcinomas histologic types.

The molecular signature of mammary lobular carcinomas is the loss of E-cadherin protein expression as evidenced by immunohistochemistry, whereas ductal carcinomas are typically E-cadherin positive.

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