Original Article

Differential expression of e-cadherin in lobular and ductal carcinoma of breast in an Iranian Cancer Care Hospital

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ABSTRACT

Objective: To determine the role of E-cadherin in differentiating Breast lobular carcinoma from Breast ductal carcinoma in Iranian patients.

Methodology: A total of 80 malignant breast samples were reviewed by two pathologists before undergoing E-cad immunohistochemistry (IHC) staining. The results from IHC were compared with the results from the morphological study of the samples and they were then analyzed statistically.

Results: The normal ductal cells had strong complete homogenous membrane E-cadherin reactivity in all cases. The complete absence of E-cadherin membrane staining occurred in 14 out of 16 cases of Lobular breast carcinoma. The mean score of E-cadherin expression in ductal carcinoma was 191.35 with a CV of 104.39. In lobular carcinoma, the mean score of E-cadherin was 9.36 with a CV of 25.68. Using the Mann–Whiteny test, the difference between E-cadherin expression score in ductal and lobular carcinoma was statistically significant (P=0.0009).

Conclusion: E-cadherin expression is a useful diagnostic tool in distinguishing ductal from lobular carcinoma of breast.

KEY WORDS: Ductal carcinoma, Lobular carcinoma, E-cadherin, Breast.

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INTRODUCTION

Breast carcinoma is the most common cancer among women, with over one million cases reported annually worldwide.[1] The incidence is 91.4/100,000 in the population of North America.[1-3]The incidence is also high in Iran, being the most common cancers among women.[4] On the basis of morphological features, these tumors are classified as ductal and lobular carcinomas.[5-6] Recognized standard histological criteria can help discriminate ductal from lobular carcinomas of the breast, however, these criteria are not unequivocal and applicable to all cases. Although morphologic features, such as Indian file, targetoid appearance, discohesiveness of tumor cells and intracytoplasmic lumen, are considered features of lobular carcinomas, they may also be present in some poorly differentiated cases of ductal carcinoma.[6]

Distinction of ductal and lobular carcinoma, especially in situ lesions, is clinically important, as their behavior, prognosis and management are quite different.[6-7] During the past several years an ever-expanding numbers of biologic, prognostic and diagnostic markers have been recorded for patients with breast cancer. E-cadherin is a member of a family of transmembrane glycoprotein responsible for

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Ca^{2+} dependent cell-cell adhesion. In epithelial cells, E-cadherin is considered one of the key molecules for the formation of the intercellular junctional complex and for the establishment of cell polarization.[8] Most researchers in this area consider E-cadherin a discriminative marker in differentiation of ductal and lobular carcinomas. The reported sensitivity and specificity varies in different studies. However, there are some conflicting results various studies correlating E-cadherin status and other prognostic and predictive factors such as size and grade of tumor, lymph node metastasis and hormone receptors.[8-10]
In this study we assessed reproducibility of IHC and morphologic criteria in discriminating between ductal and lobular carcinomas as well as the sensitivity and specificity of E-cadherin in the differential diagnosis of these tumors.

METHODOLOGY

We studied 80 breast biopsy specimens containing equal numbers of ductal and lobular carcinomas from the surgical pathology file of Cancer Institute pathology laboratory of Tehran University, Faculty of Medical Science. The specimens were collected over a five-year period between the years 2000 and 2005.

Initially, we defined characteristic lesions by morphological criteria and then performed immunohistochemical (IHC) studies. Finally, the score of E-cadherin staining of these cases was used to study the controversial lesions in order to clarify whether the differentiation was ductal or lobular.

H&E-stained slides were reviewed independently by two pathologists in order to establish a diagnosis by determining the morphology, including histological type and tumor grade on the basis of established diagnostic criteria. They were not aware of previous histological diagnosis. Discrepancies in diagnosis regarding tumor type and grade were resolved by consultation with a third pathologist.

After initial histological examination, each case was grouped according to whether the diagnosis was unanimous (agreed upon by both pathologists) or non-unanimous. Immunohistochemical studies were performed on formalin-fixed paraffin-embedded blocks. From the most representative blocks 5-µm sections were cut and deparafinized in xylene and rehydrated in graded alcohols. Sections were subjected to heat-induced antigen retrieval by heating in a 0.01 M citrate buffer (pH 6.0) for 20 minutes in a microwave. Endogenous peroxidase was blocked by 3% hydrogen peroxide in methanol for 20 minutes. Slides were then stained immunohistochemically (all reagents from Dacocytomation, Glostrup, Denmark). First, they were incubated with monoclonal antibody against E-cadherin (1:100 dilutions, NCH-38 clone) for 60 minutes. Slides were washed in buffer and incubated with link for 10 minutes. After incubation with streptavidin – HRP (LSAB 2 system HRP code K0672) for 10 minutes, slides were developed with diaminobenzidine (liquid DAB + substrate chromogen system, code K3468) for 10 minutes and counterstained with hematoxylin.

These slides were then evaluated independently by two pathologists. Controversies in evaluation were resolved by consultation with a third pathologist. Clear membranous staining was considered positive. The intensity of membranous staining was scored quantitatively on a four-tiered scale as follows:
3+: strong complete membrane staining, comparable with benign ductal or lobular epithelial cells, clearly visible on low magnification.
2+: moderate clear membrane staining visible on medium magnification.
1+: weak but still complete staining visible or high magnification.
0: absent or incomplete membrane staining.

A score generated by multiplying the intensity of staining with percentage of cells exhibiting positive staining, resulting a possible score of zero to 300. The research was carried out according to the principles of declaration of Helsinki. The local ethics review committee of Tehran University of medical science approved the study protocols.

For statistical analysis, the Mann-Whitney rank sum test and Kruskal-Wallis one-way analysis of variance by rank were used, followed by the Dunn multiple comparison test, when appropriate. The correlation between the level of E-cadherin staining and tumor type was estimated using the Spearman rank correlation test. A two-sided p value less than 0.05 was considered statistically significant.

RESULTS

Forty two cases were included in the unanimous group, agreed upon as ductal or lobular type by all pathologists. In the non-unanimous group, 38 cases were included with different opinions about the ductal or lobular nature of the lesions, as shown in Table-I. Patient age ranged from 28 to 71 years, (mean=49) with a coefficient of variation (CV) of 11.2.On reviewing the slides, 40% of the 40 cases of lobular carcinoma and 76% of cases of ductal carcinoma diagnoses were agreed upon by both study pathologists. These cases were called the unanimous group, and

the rest of the cases were called the non-unanimous group. Of all the cases, only 52.5% had complete agreement between all study pathologists.

The normal ductal cells had strong complete homogenous membrane E-cadherin reactivity in all cases. In ductal Breast carcinoma E-cadherin stained the cell membrane in 25 out of 26 cases (96.5%) with scores varying from 15-300. Only one case was negative for e-cadherin. The lobular lesions included invasive lobular carcinoma with or without an in situ component. The complete absence of E-cadherin membrane staining occurred in 14 out of 16 cases (87.5%), whereas two cases showed E-cadherin membrane staining scored 70 and 80.

The mean score of E-cadherin expression in ductal carcinoma was 191.35 with a CV of 104.39. In lobular carcinoma, the mean score of E-cadherin was 9.36 with a CV of 25.68. Using the Mann –Whiteny test, the difference between E-cadherin expression score in ductal and lobular carcinoma was statistically significant (P=0.0009).

Based on this statistically significant difference, we could calculate a score for E-cadherin expression which would give the maximum sensitivity and specificity in the differentiation between ductal from lobular carcinoma. In (Fig.2) we used a score of 7.5, which was best for this purpose. Therefore, by distribution of data the area under the receiver operating characteristic (ROC) curve (confidence interval 95% 0.787-1.011, P=0.001), the cases were classified into four groups according to their E-cadherin expression score of higher or lower than 7.5, and ductal or lobular type according to the pathologists diagnosis based on generally accepted histological criteria in H&E-stained biopsy tissue.

Using Fisher's exact test, there was a statistically significant difference between the frequency of ductal and lobular carcinoma and E-cadherin expression (P<0.005).Based on these results, we estimated the statistics for the diagnostic accuracy, including sensitivity, specificity, positive predictive value, negative predictive value, and precision, using the Wilson score method with a 95% confidence interval. We found the diagnostic accuracy of predicting lobular breast tumor histology using an E-cadherin expression level of 7.5 or lower to be: sensitivity 92.3, specificity 87.5, positive predictive value 92.31, negative predictive value 87.5 and precision 90.48. For the diagnostic accuracy of E-cadherin expression at the level or more than 7.5 for ductal breast tumor histology was: sensitivity 87.5, specificity 92.31, positive predictive value 87.5, negative predictive value 92.31 and precision 90.

We found no correlation between E-cadherin membrane staining and tumor size, tumor grade, tubule formation, nuclear pleomorphism, mitotic activity or axillary lymph node status (Table-II).

Table-I: Frequency of ductal and lobular carcinoma in primary reports.

Pathology	*Frequency*	*Relative frequency (%)*
Lobular carcinoma	40	50
Ductal carcinoma	34	42.5
Consistent with ductal carcinoma	3	3.75
Consistent with lobular carcinoma	2	2.5
Invasive carcinoma	1	1.25
Total	80	100

DISCUSSION

The traditional classification of infiltrating breast carcinomas into ductal and lobular can be diagnostically challenging in a small proportion of cases with equivocal histological features and in in-situ lesions with overlapping features.[10]

Distinguishing between the infiltrating ductal (IDC) and lobular (ILC) carcinomas is clinically important because of the different pattern of systemic metastases and prognostic evaluation.[10] Invasive

Table-II: Log-rank test differences in E-cadherin expression among 80 patients with breast ductal or lobular carcinoma.

Characteristics		*Mean score*	*CV*	*P value*
Size of tumor all cases (n=80)	T1=24	119.79	119.05	
	T2=46	116.3	117.88	0.31
	T3=66.5	66.5	92.14	
Size of tumor (n=24) ductal, T unanimous	T1=6	185	118	
	T2=18	(93.68)	102.33	0.85
	T3=0			
Size of tumor (n=16) lobular unanimous	T1=5	16	37.58	
	T2=8	0	0	0.31
	T3=3	23.33	40.41	
Lymph node (n=80) status All cases	M+=47	122.33	116.99	
	m-=33	94.45	112.81	0.31
Lymph node (n=24) status Ductal, unanimous	M+=14	190.35	109.59	
	m-=12	192.5	101.81	0.69
Lymph node (n=16) status Lobular, unanimous	M+=8	8.75	24.75	
	m-=8	10	28.28	0.93
Grade of tumor ductal unanimous	I=5	220	63.64	
	II=46	220	92.72	0.54
	III=3	145	134.35	

lobular carcinoma (ILC) is the second most common type of invasive breast cancer after Invasive ductal carcinoma, and accounts for 5-15% of all breast cancer cases.[11]

Lobular carcinoma has been shown to have a higher incidence of multicentricity and bilaterality in the breast than ductal carcinoma. Distinctive patterns of systemic metastases are also seen with invasive lobular carcinoma. Metastases to the peritoneum and retroperitoneum, leptomeninges, gastrointestinal tract, and gynecologic organs are seen at a higher frequency in lobular carcinomas than ductal carcinomas. The frequency of metastases to lung and pleura are lower in lobular than ductal carcinomas.[12]

Thus, lobular carcinomas, needs to be differentiated from ductal carcinomas. When the cytologic of lobular carcinoma approaches to that of ductal this differentiation may be difficult. The problem may be compounded when the ductal carcinoma exhibits a dispersed infiltrating pattern.[12]

E-cadherin (E-CD) is a member of a family of transmembrane glycoproteins. In epithelial cells, E-cadherin is involved in the formation of intercellular junctional complexes, and decreased expression of E-cadherin is thought to be associated with invasiveness of tumor cells.[10-11] Cell-cell and cell-matrix adhesion molecules currently comprise five distinct groups, i.e. integrins, selectins, the CD44 group, the immunoglobulin family of receptors, and cadherins. Cadherins are a multigene, multifamily of transmembranous, cell-cell adhesion receptor molecules; they are characteristically homophilic, ie each molecule selectively binds to an identical molecule in a neighboring cell. E-cadherin is the archetypical, epithelial representative of this family; the locus of concentration of E-CD in the cell membrane is at the adherence junctions.[11-12]

We studied 80 cases of breast carcinoma and found disagreement in diagnosis in 60% of cases originally diagnosed as lobular carcinoma. Nurismah et al. and Wahed et al. showed E-CD is a useful marker to differentiate between IDC and ILC of the breast (same our study).[10,12]

Our studies largely share results with many others already conducted in other countries. Therefore, the race and endemic specifications of patients are unlikely to affect E-cad market in breast cancer.

Wahed et al. also showed E-CD is useful in differentiate Pleomorphic Lobular carcinoma from IDC.[12] Bratthauer et al. and Lehr et al. showed combination of E-CD and High molecular weight Cytokeratin immunoprofile(CK8) are extremely useful in distinguishing lobular and ductal lesion and clarifying the nature of some of the morphologically intermediate cases.[13-14]

In view of the results from the study, we can use some IHC markers like CK8 to offer a better analysis of morphologically undifferentiated lobular and ductal malignancies. Due to the high incidence of Breast carcinoma in our country and even the world and given the clinical and treatment differences of their different morphological cases, it is very significant to differentiate them. A definite diagnosis of the morphology of the cancer allows a better treatment strategy, not to mention lower healthcare costs. Given the expensive treatment of breast cancer, it would be much more cost-effective to carry out IHC notably in developing countries.

We offer a large study to evaluate the correlation between E-Cadherin and tumor grade to estimate its prognostic potential. In conclusion E-cadherin membrane staining reliably distinguished ductal and lobular carcinoma including problematic cases that were difficult to classify using H&E-stained slides. We recommend E-cadherin staining routinely on problematic cases, especially when the classification has therapeutic implication.

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