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Original Research Article

Significance of CK 8 and E-Cadherin in Differentiating Lobular From Ductal Breast Carcinoma and its Correlation with Clinicopathological Parameters

Bharti Kumari[1], Mamta Kumari[2], Debaditya Haldar[3], Shashi Singh[4], Amresh Kumar[5]

[1]Assistant Professor, Department of Pathology, IGIMS, Patna
[2]Assistant Professor, Department of Pathology, IGIMS, Patna
[3]Junior Resident, Department of Pathology, IGIMS, Patna
[4]Associate Professor, Department of Surgical Oncology, IGIMS, Patna
[5]Junior Resident, Department of Pathology, IGIMS, Patna

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Corresponding Author: Dr. Mamta Kumari
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Abstract:

Introduction: Differentiating the cases of invasive ductal carcinoma (IDC) from invasive lobular carcinoma (ILC) is difficult in equivocal cases due to some overlapping features. In such cases E-cadherin and Cytokeratin 8 (CK 8) immunohistochemical markers can be useful in confirming the results.

Aim & Objectives: To explore E-cadherin and CK 8 expression in breast carcinoma cases having ductal and lobular morphology, and to determine the role of these two immunohistochemical markers in distinguishing between IDC and ILC and their in-situ components, and to correlate with clinicopathological parameters.

Methods: We conducted a prospective observational study on 80 breast carcinoma cases in the Pathology Department of IGIMS, Patna over a period of one year, from 2022 to 2023. Haematoxylin & Eosin stained slides of tissue samples of breast carcinoma cases were studied for histomorphological parameters and manual immunohistochemistry was performed with E-cadherin and CK 8 to evaluate its expression and correlate it with histomorphological findings.

Results: There were 65 IDC and 15 ILC cases. We found Ductal carcinoma in situ (DCIS) in 40 cases and Lobular carcinoma in situ (LCIS) in 10 patients. There was significant statistical correlation of E-cadherin score and CK 8 expression pattern in IDC, ILC, DCIS and LCIS.

Conclusion: The combination of CK 8 and E-cadherin immunohistochemical markers could be used as diagnostic utility markers in cases of IDC, ILC, DCIS and LCIS.

Keywords: E-cadherin, Cytokeratin 8, Immunohistochemistry, Breast.

Introduction

Worldwide, breast cancer is the commonest cancer among women globally. To give accurate diagnosis and outline treatment plans, there is need for differentiating between invasive lobular carcinoma (ILC) and invasive ductal carcinoma (IDC). Although we have defined histopathological features for IDC and ILC, we still face problems in case of poorly differentiated carcinoma (PDC) and pleomorphic variant of ILC [1].

In addition, lobular carcinoma in situ (LCIS) also shares some similar features with low grade ductal carcinoma in situ (DCIS) [2]. In such a situation, E-cadherin and Cytokeratin 8 (CK 8) are novel immunohistochemical markers which can help in differentiating these close entities.

E-cadherin is responsible for cell to cell adhesion [3]. Most ILC and LCIS show complete loss of E-cadherin expression, whereas E-cadherin expression is retained in IDC and DCIS [4]. CK 8 shows predominantly peripheral membranous staining pattern in IDC, whereas ILC shows perinuclear ring-like cytoplasmic staining [5]. Making a clear cut distinction between ILC and IDC is very important as they behave quite different clinically and prognostically [6,7]. So, we conducted this study to analyse E-Cadherin and CK 8 expression in ILC and IDC, and to determine its role in distinguishing between the two and also their in-situ components. We also tried to study the correlation between the clinicopathological parameters and E-cadherin and CK 8 expression.

Materials and Methods

We conducted a prospective observational study on 80 cases of breast carcinoma at the Department of Pathology, IGIMS, Patna over a period of one year,

from 2022 to 2023. All histologically confirmed primary breast carcinoma cases were included in our study.

However, we excluded those breast carcinoma cases which were received from patients who had undergone neoadjuvant chemotherapy, and those having recurrent breast carcinoma.

Methodology

Haematoxylin and eosin stained slides of tissue samples (biopsy specimens and modified radical mastectomy cases) of breast carcinoma were studied for histomorphological parameters and manual immunohistochemistry was performed with E-cadherin and CK 8 to evaluate its expression and correlate with histomorphological findings. The primary antibody for E-cadherin was monoclonal mouse antihuman antibody, clone 36 (X-BioGenex) and clone C51 for CK 8.

E cadherin scoring was done are as follows:

- 3+ score was awarded for strong intermembranous staining in most of tumor cells;
- 2+ score given for moderate intermembranous staining in >10% of tumor cells;
- 1+ score indicated weak intermembranous staining in <10% of tumor cells; and a score of zero depicted absence of intermembranous staining in tumor cells.

CK 8 showed predominantly peripheral membranous staining pattern in IDC cases and perinuclear ring-like cytoplasmic pattern in ILC. A written informed consent was obtained from each participant before enrolling them in the study and prior approval was taken from the Institutional Ethics Committee (456/IEC/IGIMS/2022).

Statistical Analysis was done using SPSS software, version 21.0. Chi-square test was performed and p-value less than 0.05 was considered as statistically significant.

Result

Out of the 80 confirmed breast carcinoma cases in our study, 60 specimens were small biopsies and 20 were modified radical mastectomy specimens. 65 cases were diagnosed as IDC and 15 cases as ILC. DCIS was noted in 40 cases and LCIS in 10 cases. Table 1 demonstrates the relationship of E-cadherin expression with clinicopathological parameters like age, tumor size, tumor grade, focality, lymphovascular invasion (LVI), perineural invasion (PNI), axillary lymph node involvement, nipple-areolar complex involvement, overlying skin involvement and base involvement. Correlation of CK 8 expression pattern with clinicopathological variables are highlighted in Table 2. Relationship of E-cadherin score and CK 8 expression pattern with IDC, ILC, DCIS and LCIS have been demonstrated in Tables 3, 4, 5 and 6.

Table 1: Relationship of E-cadherin expression with clinicopathological parameters

Parameters		Frequency	E-cadherin score				p-value
			3+	2+	1+	0	
Age (years)	<50	35	20	9	4	2	0.9
	≥50	45	26	10	6	3	
Tumor size (in cm)	<2	5	2	1	1	1	0.8
	2-5	5	1	2	1	1	
	>5	10	6	1	1	2	
Grade	1	10	7	1	1	1	0.05
	2	50	36	12	1	1	
	3	20	8	6	4	2	
Focality	Unifocal	10	3	1	3	3	0.25
	Multifocal	10	2	5	2	1	
LVI	Present	15	8	4	2	1	0.7
	Absent	65	26	22	9	8	
PNI	Present	5	1	1	2	1	0.05
	Absent	75	30	25	19	1	
Axillary Lymph nodes	Involved	15	8	3	4	1	0.2
	Not involved	5	1	1	1	2	
Nipple areola complex	Involved	5	2	1	1	1	0.6
	Not involved	15	9	4	1	1	
Overlying skin	Involved	5	1	1	1	2	0.2
	Not involved	15	8	4	2	1	
Base	Involved	6	2	2	1	1	0.7
	Not involved	14	8	4	1	1	

Table 2: Relationship of CK 8 expression pattern with clinicopathological parameters

Parameters		Frequency	CK8 (peripheral)	CK8 (perinuclear)	p value
Age (years)	<50	35	30	5	0.3
	≥ 50	45	35	10	
Tumor size (in cm)	<2	5	3	2	0.7
	2-5	5	4	1	
	>5	10	7	3	
Grade	1	10	5	5	0.003
	2	50	46	4	
	3	20	15	5	
Focality	Unifocal	10	4	6	0.06
	Multifocal	10	8	2	
LVI	Present	15	8	7	0.0009
	Absent	65	58	7	
PNI	Present	5	3	2	0.95
	Absent	75	46	29	
Axillary Lymph nodes	Involved	15	10	5	0.7
	Not involved	5	3	2	
Nipple areola complex	Involved	5	3	2	1.0
	Not involved	15	9	6	
Overlying skin	Involved	5	3	2	0.5
	Not involved	15	11	4	
Base	Involved	6	3	3	0.2
	Not involved	14	11	3	

Table 3: E-cadherin score in IDC and ILC

E-cadherin score	IDC (n=65)	ILC(n=15)	p value
3+	50	1	0.004
2+	10	1	0.68
1+	5	3	0.35
0	0	10	0.00001

Table 4: E-cadherin score in DCIS and LCIS

E cadherin score	DCIS (n=40)	LCIS (n=10)	p value
3+	30	1	0.044
2+	5	1	1.0
1+	3	1	1.0
0	2	7	0.001

Table 5: CK 8 peripheral membanous staining (PE) and perinuclear ring-like cytoplasmic staining (PN) in IDC and ILC

CK 8 expression pattern	IDC(n=65)	ILC((n=15)	p value
PE	60	3	0.02
PN	5	12	0.0001

Table 6: CK 8 peripheral membranous staining (PE) and perinuclear ring-like cytoplasmic staining (PN) in DCIS and LCIS

CK 8 expression pattern	DCIS(n=40)	LCIS(n=10)	p value
PE	33	2	0.06
PN	7	8	0.01

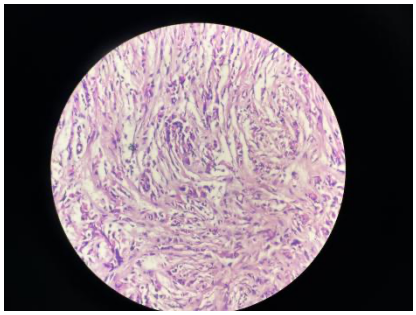

Figure 1: showing Invasive ductal carcinoma (40x: H & E)

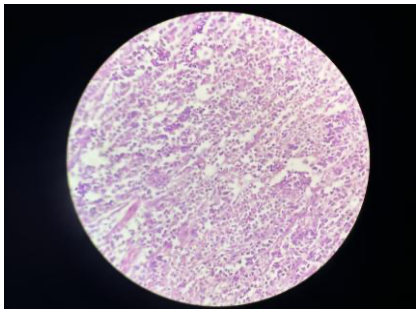

Figure 2: showing Invasive lobular carcinoma, typical Indian file pattern (40x: H & E)

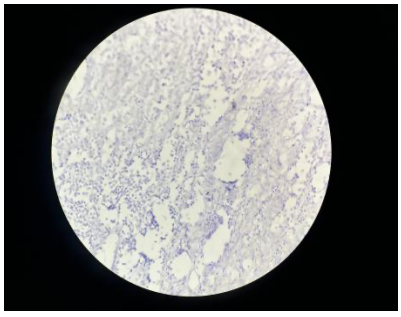

Figure 3: showing E cadherin score -0 in Invasive lobular carcinoma (40x)

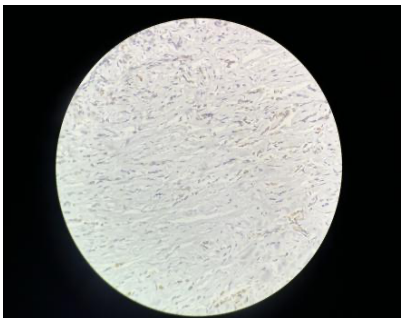

Figure 4: showing E cadherin score-2+ in Invasive lobular carcinoma (40x)

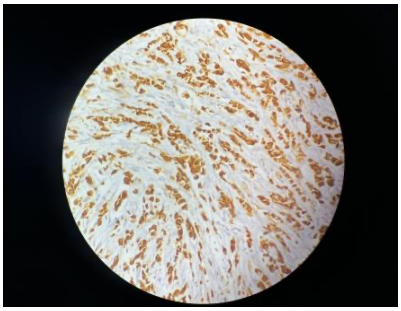

Figure 5: CK 8 showing membranous staining pattern in Invasive ductal carcinoma (40x)

Discussion

The term LCIS was coined by Foote and Stewart for those breast carcinoma cases having monomorphic intralobular proliferation of cells [8].

Although ILC shows characteristic Indian file pattern of small tumor cells (Figure 2) with little nuclear pleomorphism, its variants like solid, alveolar, tubulo-lobular and pleomorphic subtypes pose a problem as they morphologically mimic IDC of no special type (Figure 1). ILC shows loss of E-cadherin expression. E-cadherin is emerging as a novel marker, however researchers have also found that E-cadherin expression in some ILC cases should not preclude its diagnosis [9].

Our study showed a significant correlation of E-cadherin expression with IDC and ILC [Table 3]. 50 out of 65 IDC cases showed 3+ E-cadherin score with a p-value 0.004. 10 out of 15 ILC cases showed absence of E-cadherin staining with a p-value of 0.0001. With respect to in-situ lesions [Table 4], out of 40 cases of DCIS, 30 showed 3+ E-cadherin positivity with a p-value of 0.044. Out of 10 cases of LCIS, 7 showed no E cadherin expression (Figure 3) with a significant p-value of 0.001.

Wiljo et al in his study analysed 48 IDC and 38 ILC cases and showed that all the 48 IDC had E-cadherin expression while 32 out of 38 ILC cases (84%) showed complete loss of E-cadherin [10]. Gamallo et al and Moll et al revealed that majority of ILC show a complete loss of E-cadherin, whereas all IDC cases retain E-cadherin expression [11,12]. Our study results were equivalent with the above studies. R Singhai et al concluded in the year 2011 that loss of E-cadherin expression confirmed the diagnosis of ILC with 97.7% specificity and 88.1% sensitivity [13]. Gamallo et al and Moll et al also revealed that some cases of IDC showed reduced expression of E-cadherin emphasizing their poor differentiation and association with high histological grade [11,12].

IDC and ILC show similar growth in a targetoid pattern around benign ducts in some cases creating diagnostic dilemma. In such cases, positive E cadherin expression aids in the diagnosis of IDC. R Singhai et al also emphasized in his study that the breast carcinoma cases previously diagnosed as invasive cancer of uncertain type could be reclassified with the help of E-cadherin staining into ILC and IDC.

In addition, variants of ILC like tubulo-lobular, solid, pleomorphic and signet ring cell subtypes also pose diagnostic difficulties with IDC [14,15]. In our study, 3 tubulo-lobular variants of ILC showed 1+ score and 1 solid variant showed 2+ score (Figure 4). 1 pleomorphic variant showed 3+ E-cadherin score. These findings were in contrast to previous studies where loss of E-cadherin expression has been used as a reliable marker to distinguish ILC variants from IDC.

CK 7 and CK 8 have been utilized in the identification of breast carcinoma, and even these cells are present in very small numbers [16]. CK 7, 8, 18 and 19 are expressed in breast carcinomas of ductal and lobular morphologies. CK 8 shows a predominantly peripheral membranous staining pattern in IDC (Figure 5) while perinuclear ring-like cytoplasmic staining pattern seen in ILC. Our study showed 60 out of 65 IDC cases having peripheral predominant membranous staining pattern with a p-value of 0.02. 12 out of 15 ILC cases showed perinuclear ring-like cytoplasmic pattern with a p-value of 0.0001 [Table 5]. 7 out of 40 DCIS cases and 8 out of 10 LCIS cases showed perinuclear pattern [Table 6] with a significant correlation, p-value 0.01. Schaller et al found that luminal marker expression was associated with good prognosis whereas expression of basal markers (CK 5/6) was associated with a poor prognosis [17]. Aiad et al conducted a study on 70 breast carcinoma patients and concluded that there was abnormal expression of CK8/18 in 70% cases. This is mainly due to the presence of tumor heterogeneity in CK expression worldwide. M Becker et al in his study proposed that loss of CK 8/18 expression or a low CK 8/18 expression was associated with poor prognosis [18]. There was, however, no significant correlation of E-cadherin expression with clinicopathological parameters like age, tumor size, tumor grade, focality, LVI, PNI, axillary lymph node involvement, nipple areola complex involvement, overlying skin involvement and base involvement [Table 1].

On the other hand, there was significant association of CK 8 expression pattern with histological grade of the tumor and lymphovascular invasion [Table-2]. This finding is a new thing in our study which is yet to be explored in future.

Limitations

We used manual method for immunohistochemical staining in our setup. This could be the reason for discrepant results in interpretation apart from aberrant expression pattern of CK 8 and E-cadherin. Additional shortcomings included the lack of a validated specific antibody clone, and different reagents used.

Conclusion

There was significant association of E-cadherin and CK 8 expression in IDC and ILC. So, the combination of CK 8 and E-cadherin immunohistochemical markers could be used as diagnostic utility markers in cases of IDC, ILC, DCIS and LCIS. One new thing that we found in our study was that there was significant statistical correlation of CK 8 expression pattern with tumor

grade and lymphovascular invasion. The correlation of E-cadherin expression with above mentioned clinicopathological parameters is yet to be explored in near future on a large scale.

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