Aberrant expression of E-cadherin in infiltrating ductal and lobular breast carcinomas and its correlation with clinicopathological parameters – A hospital-based study

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DOI: https://doi.org/10.17511/jopm.2021.i03.02

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Introduction: Breast carcinoma is one of the commonest malignant tumours in women, leading to premature deaths and morbidity. E-cadherin is a 120kDa calcium-dependent transmembrane glycoprotein encoded by the CDH1 gene located on chromosome 16q21 and is expressed in most epithelial cells. Loss of E Cadherin expression implies cell discohesion and favours metastasis. **Materials and Methods:** A total of 30 cases of breast carcinomas were studied, over two years. Histological grade and type were assessed by staining the paraffin-embedded sections with H & E. Using IHC technique, E-cadherin antigen was retrieved by Heat-Induced Epitome Retrieval method, and immunostaining was scored semiquantitatively. Cases were grouped as 'preserved,' when positivity was strong membranous, and occurred in more than 75% of the neoplastic epithelial cells and 'aberrant' in all the remaining cases. **Results:** E-cadherin was found to be preserved in 46.7% of all the breast carcinomas and aberrant in 51.7% of invasive ductal carcinomas (IDC) alone, while 100% of invasive lobular carcinomas showed aberrant expression. No significant correlation was found with E-cadherin grading and histological type of carcinoma, histopathological grade or involvement of deep surgical margin. **Conclusion:** Differentiation between invasive ductal and invasive lobular carcinoma based on the loss of E-cadherin has to be done cautiously given its aberrant expression in ductal carcinomas as well.

Keywords: Invasive Ductal Carcinoma (IDC), Invasive Lobular Carcinoma (ILC), E-cadherin, IHC, Grading of E-cadherin, Aberrant E-cadherin expression, Breast carcinoma

Introduction

Breast carcinomas are the most common of the malignant tumours and the leading cause of cancer-related death in women with more than one lakh cases occurring worldwide annually [1]. It accounts for 25-32 percent of all cancers in women in India and 12.5 percent worldwide[2]. The important morphologic prognostic factors in invasive carcinoma of the breast include the size of the primary tumour, microscopic grade, axillary lymph node metastasis, blood and lymph vessel emboli, tumour necrosis, skin invasion and nipple invasion[3]. Other possible prognostic parameters like cell proliferation index, estrogen receptor, progesterone receptor, Her2 neu receptor status, p53, bcl-2 are of growing interest [4]. Some studies have shown an independent prognostic significance of histologic grade in breast cancer[5-7] although grading is a subjective evaluation that may have problems with reproducibility[8,9].

The grading of breast cancer was first suggested in 1957 by Bloom and Richardson which was based mainly on architectural features. It was modified by Nottingham and the grade is now obtained by adding up the score for the tubule formation, nuclear pleomorphism, mitotic count.10 The Nottingham modification of the Scarff- Bloom- Richardson (NSBR) grading system provides a platform for uniformity in grading[10,12]. The Nottingham Prognostic Index (NPI), is calculated from three parameters namely tumour size, lymph node stage and histologic grade[13]. It predicts prognosis and helps to distinguish groups of patients suitable for different forms of therapy[13].

The mechanism of progression is not completely recognised. Research suggests that the extracellular part of cadherin, via the ions of calcium, influences catenin with similar molecules on the neighbouring cell. Thus, they create an adhesive complex that maintains cells in compact connection [14]. The cancerous process results in the reduction of the amount of cadherin, which in turn results in disorder in the transition of intracellular signals, and loosened intercellular connections. This leads to increased cells' invasiveness and ability to migrate[15]. Previous data confirm that diminished E-cadherin expression is seen in patients suffering from breast, lung, prostate and stomach cancer[16]. and this correlated with histological grading/reduced variations/of cancer, presence of distant metastasis, and worse prognosis [17].

The results estimating E-cadherin in patients with breast cancer are contradictory. In most of them, it was proved that the loss of E-cadherin expression was a bad prognostic factor[18] and was connected with a higher level of malignancy, metastasis in axillary lymph nodes, absence of estrogen and progesterone receptors, and presence of recurrence[19]. The objective of this study is to study analyse the E-cadherin expression in both infiltrating ductal and lobular carcinomas of the breast and to determine its usefulness in distinguishing one from the other.

Materials and methods

Setting: Department of Pathology, Jawaharlal Nehru Medical College, Belagavi.

Duration and type of study: A cross- sectional study conducted for a period of two years from January 2014 to December 2015.

Sampling methods: This study was conducted on 30 patients who were histologically diagnosed as either infiltrating ductal carcinoma or infiltrating lobular carcinoma.

Sample size: 30 cases

Inclusion criteria: Histologically proven carcinoma of the breast

Exclusion criteria: Breast cancer specimens received:
- From patients who have undergone chemotherapy for breast carcinoma
- From patients with recurrent carcinoma breast
- Where there are no sufficient viable tumour cells for the accurate evaluation of the immunohistochemical results.

Sample Collection procedure: The specimens were thoroughly examined and clinical details were analysed. The specimen sent in formalin was sliced at 1cm intervals and fixed immediately in 10% NBF (Neutral Buffered Formalin). One dedicated block from the tumour, fixed in formalin for not more than 24 hours, was used for IHC. Four-micron thickness sections were cut and taken on amino silane coated slides and stained for evaluating E- Cadherin expression. Also, the sections were routinely stained with H & E and histological grading of the tumour was done on these sections according to Modified Bloom- Richardson grading.

Table-1: Histological grading using Nottingham modification of Scarff Bloom Richardson system [10].

Criteria	Score- 1	Score- 2	Score- 3
Tubule formation	>75% of tumour	10-75% of tumour	<10% of tumour
Nuclear pleomorphism	Minimal variation in size and shape of nuclei	Moderate variation in size and shape of nuclei	Marked variation in size and shape of nuclei
Mitotic count per 10 hpf (0.44mm) filled diameter	0-5	6-10	>11

Table-2: The scores are of the histological grading[10]

Grades	Scores
Grade 1	3 – 5
Grade 2	6, 7
Grade 3	8, 9

Scoring of E-cadherin: The procedure followed for IHC staining is according to standard guidelines. IHC stained sections were observed under light microscopy and the staining was scored. E-cadherin staining interpretation has been adapted from a study done by Esposito N and David J Dabbs, published in Modern Pathology in 200724.

- The immunoreactivity with E-cadherin was scored semiquantitatively as follows:

- Strong inter-membranous staining in >75%= 3+; moderate staining in 26- 75%= 2+, weak staining in 1-25%= 1+, absence of membranous staining= 0 Cases were grouped as 'preserved,' when positivity was strong membranous, and occurred in more than 75% of the neoplastic epithelial cells and 'aberrant' in all the remaining cases, including the ones where staining was absent[26].

Statistical analysis: It was done using "R" software and the relationship between clinicopathological parameters, IHC grading, and histopathological grading was established using Fischer exact test.

Results

In the present study, age ranged from 32 to 70 years and the mean age ± SD was 49.7± 10.29 years. Majority, 10 cases (33.4%) occurred in 41-50 years age group followed by 9 (30%) in 51-60 years, 7(23.3%) in 31-40 years, and 4(13.3%) in 61-70 years age group (Table – 3).

29 cases (97%) were women and one case (3%) was a 67-year-old man. 24 patients (80%) presented with breast lump alone, followed by breast lump associated with nipple retraction in 3 patients (10%), breast lump with nipple discharge in 2 patients (6.7%) and only one patient (3.3%) had breast lump associated with pain.

One case (3.3%) had a family history of carcinoma. The size of tumor measured between 2.0 - 5.0 cms in 14 cases (46.7%), followed by ≤ 2 cms in 9 cases (30%) and > 5 cms in 7 cases (23.3%).

Table 3: Age distribution of carcinoma breast

Age in years	Number of patients	%
31-40	7	23.3
41-50	10	33.4
51-60	9	30
61-70	4	13.3
Total	30	100

Mean age ± SD = 49.7± 10.29

Table – 4: Scores of histological grade: (Elston and Ellis modification of Scarff- Bloom- Richardson grading system)

Score	Tubule formation	Nuclear pleomorphism	Mitotic counts
1	1 (3.3%)	0 (0%)	27 (90%)
2	17 (56.7%)	12 (40%)	3 (10%)
3	12 (40%)	18 (60%)	0 (0%)
Total	30	30	30

In the current study, 17 cases (56.7%) had a score of 2, 12 (40%) had a score of 3 and one cases (3.3%) had a score of 1 for tubule formation. Concerning nuclear pleomorphism, eighteen cases (60%) had a score of 3, 12 (40%) has a score of 2 and none had a score of 1.

Twenty-seven cases (90%) had score 1, 3 (10%) had score 2 and none had a score of 3 of mitotic count (Table – 4).

Table-5: Histological grade in various types of carcinoma breast (Elston and Ellis modification of the Scarff- Bloom- Richardson grading system)annexure V

Histological grade	No. of patients	%
Grade 1	7	23.3
Grade 2	21	70
Grade 3	2	6.7
Total	30	100

In the present study, 21 cases (70%) were of histological grade 2, followed by 7 cases (23.3%) of grade 1 and 2 cases (6.7%) of grade 3 (Table – 5).

The predominant histologic subtype was infiltrating ductal carcinoma (NOS) amounting to 27 cases (90%), followed by 2 cases (6.7%) of intracystic papillary carcinoma and one case (3.3%) of invasive lobular carcinoma.

Table-6: Clinical and Gross features in 27 cases of IDC NOS

	Number (n=27)	%
Gender distribution		
Female	27	100
Male	0	0
Gross appearance		
Nipple retraction	3	11.1
Peaude orange	0	0
Ulceration	1	3.7
Cut surface		
Infiltrating	26	96.3
Circumscribed	1	3.7
Nodular	0	0
Diffuse	0	0
Consistency		
Firm	26	96.3
Gritty	1	3.7

Table-7: Microscopic features of 27 cases of IDC-NOS

Histological grade	No. of cases	%
Grade 1	6	22.2
Grade 2	19	70.4
Grade 3	2	7.4
Other features		
DCIS component	5	18.5
Necrosis	9	33.3
Hemorrhage	4	14.8
Calcification	0	0
Osseous metaplasia	1	3.7
Lymphocyte reaction	7	25.9
Lymohovacular invasion	9	33.3
Deep surgical margin involved	4	14.8
Lymphnode status		
Positive	12	44.4
Negative	10	37
Not- available	5	19

Of the 27 IDC NOS specimens, 19 were of MRM, 7 were of lumpectomy, and one was of TRM. In the majority of the cases, the cut surface showed a grey-white tumour with infiltrating border and firm to hard in consistency (Table – 6). Histologically, 19 cases (70.4%) are grade 2 tumours, followed by 6 (22.2%) of grade 1, and 2 (7.4%) of grade 3.

Necrosis and haemorrhage were seen in 9 (33.3%) and 4 cases (14.8%) respectively. Calcification was not seen in any of these cases. In one case (3.7%), osseous metaplasia was observed. Insitu component was noticed in 5 (18.5%) of cases, with comedo necrosis as the most common pattern. Four cases (14.8%) showed involvement of deep surgical margin. Twelve out of 25 cases in which lymph nodes were available showed metastasis and the rest showed reactive changes (Table – 7).

Out of 30 cases, 2 were IDC variant of intracystic papillary carcinoma and one was invasive lobular carcinoma, of which one intracystic papillary carcinoma was seen in a 67-year male. Both the papillary carcinomas had shown microcalcification histologically, and no involvement of the lymph nodes. Whereas, one case had shown an association with in situ component and necrosis while the other had a lymphovascular invasion. The case of invasive lobular carcinoma showed lymphocytic infiltration, Indian file pattern, targetoid pattern, lymphovascular invasion but no necrosis, haemorrhage or any association with in situ component was noted. The lymph nodes showed metastatic deposits.

Male breast carcinoma: 1 case (3.3%)In the present study, one case of intracystic papillary carcinoma was seen in a 67-year-old male patient. The specimen was of MRM which showed a grey-white tumour with infiltrating border. Histologically, the tumour was of grade 1 and showed large round to oval cells arranged predominantly in papillary fashion, also in groups, cords and tubules at places, separated by fibrous tissue. Lymphovascular invasion was present, though the lymph nodes did not show any metastatic deposits.

Table-8: Lymph node status in various types of carcinoma breast

Lymph node status	No. of patients (n=30)	%
Positive	13	43.3
Negative	12	40
Not available	5	16.7

In the current study, 13 cases (43.3%), showed nodal metastasis, 12 (40%) were negative for tumour deposits while in 5 (16.7%) cases, nodes were not available for examination (Table – 17).

Immunohistochemical profile: In the present study, 14 cases (47%) showed +3, E-cadherin grade, 7 (23%) showed +2, 8 cases(27%) showed +1, and one case (3%) showed grade 0

Immunostaining (Table – 17) IHC scoring: The interpretation of E-cadherin immunostaining was adapted from a study done by Esposito and David Dabbs [24].

Table-9: Interpretation of E-cadherin

| Grade	No. of cases (n=30)	%
+3	14	46.7
+2	7	23.3
+1	8	26.7
0	1	3.3
Total	30	100

In the present study, 14 cases (46.7%) showed +3 E-cadherin grade, i.e, strong intermembranous expression in > 75% of tumor cells, 7 cases (23.3%) showed +2, i.e, moderate staining in 26-75 % of cells, and 8 cases (26.7%) showed +1, i.e, weak membranous staining in < 25 % of tumor cells. One case (3.3%) was invasive lobular carcinoma which showed a complete absence of membrane staining (Table – 9). Grade of +3 (46.7%) was considered as a preserved expression of E-cadherin while +2, +1 and 0 grades, were considered as aberrant expression of E-cadherin, which accounted for 16 cases (53.3%).

**Graph – 1: Correlation between the E-cadherin immunostain grading and the lymph node status**
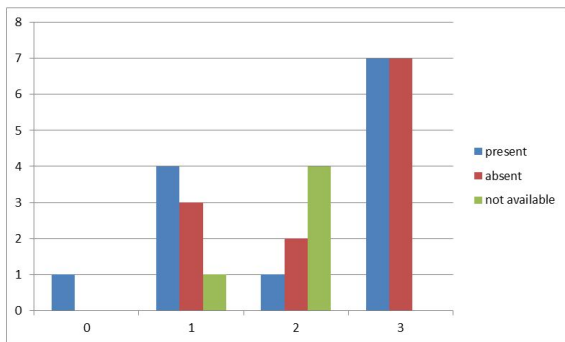
In this study, 1 (3.3%) case showed 0 grade, 8 (26.7%) cases showed + 1, 7 (23.3%) showed + 2 and 14 (46.7%) showed + 3E-cadherin immunostain. The case which showed 0 grade had a positive lymph node. Of the 8 cases (26.7%) of +1, 4 showed lymph node positivity, of the 7 cases of +2 grade, only 1 showed positivity and of 14 cases (46.7%) of +3, 7 showed positive and 7 showed negative lymph nodes. In 5 cases, lymph nodes were not retrieved. The p-value was at 0.046, hence not correlating with the E-cadherin loss (graph 1).

Graph-2: Correlation between E-cadherin grading and the histopathological grade
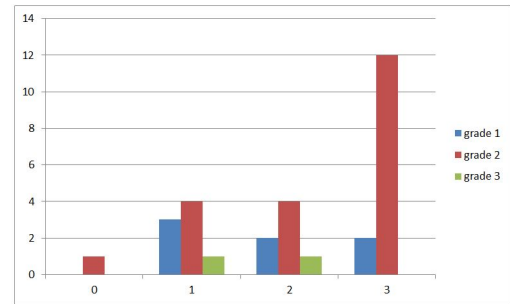

In this study, one case (3.3%) of grade 0 E-cadherin was of histopathological grade 2. Of the eight cases (26.7%) of +1, 3 were grade 1, four were grade 2 and 1 was grade 3. Of the 7 (23.3%) cases of +2 E-cadherin grade, 2 were grade 1, 4 were grade 2, and one was grade 3. Of the 14 cases (46.7%) of +3 immunostain, 2 were grade 1, 12 were grade 2. There was no correlation with the E-cadherin immunostaining with that of the histopathological grade as the p-value was 0.405 which is not significant. (graph 2).
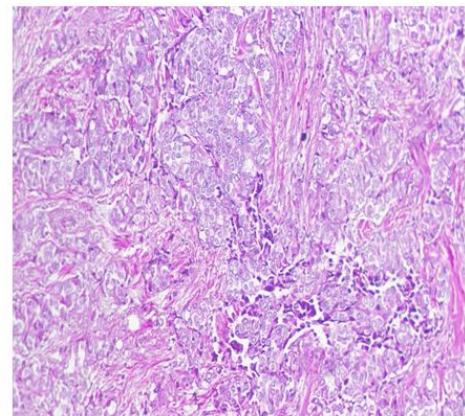
Photomicrograph 1:
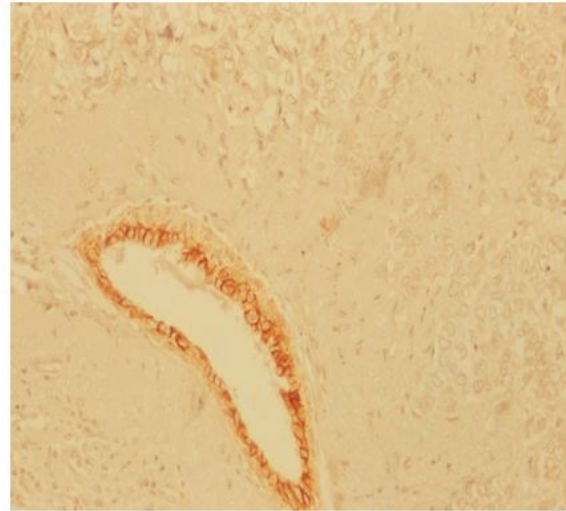Showing invasive ductal carcinoma – Grade 3 (H & E, x10)
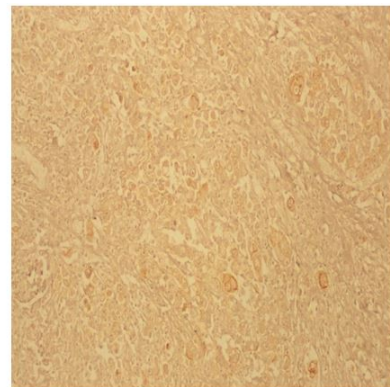
Photomicrograph 2:

Showing mild membranous staining of E-cadherin in <25%

tumour cells, in invasive ductal carcinoma –
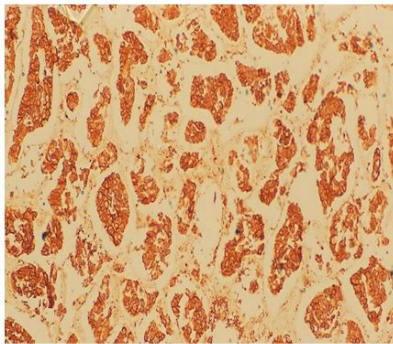
E-cadherin grade +1 (IHC, x10)
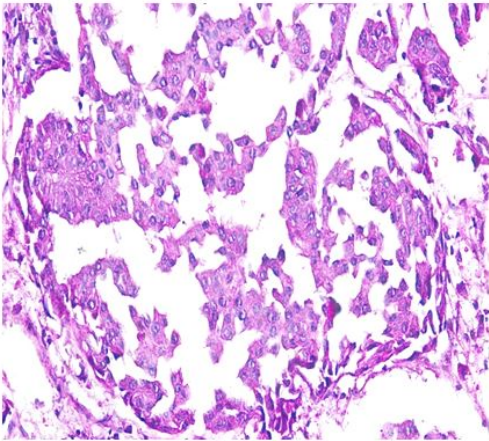
Photomicrograph 4 :

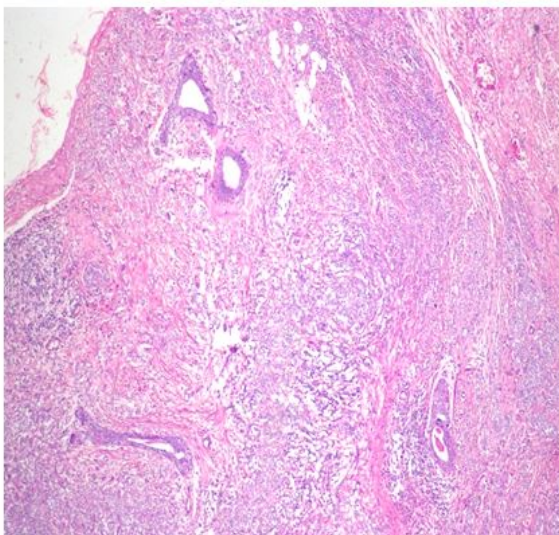Showing invasive ductal carcinoma – Grade 2 (H & E, x 20)

Photomicrograph 5:

Showing strong inter -membranous staining of E-cadherin in > 75% of tumour cells, in invasive ductal carcinoma –E-cadherin grade +3 (IHC, x 10)

Photomicrograph 10:
Showing targetoid pattern in invasive lobular carcinoma – Grade 2 (H & E, x 10)

Photomicrograph 12 :
Showing absence of E-cadherin staining in invasive lobular carcinoma - E-cadherin grade - 0 (IHC, x 10)

Discussion

In the present study, mean age was in concordance with the study conducted by Munjal. K et al.[27] and was lesser when compared to the other studies done by Amboise et al [28]. and Kowalski et al [26]. The mean age of Indian breast cancer patients is found to be lower when compared to the western countries with an average difference of 5 years. In the study conducted by Raina V et al.[29]. 7% of the carcinoma breast cases had a first degree relative with a history of breast cancer. In the present study, there is only one case of carcinoma breast with a family history, which accounts for 3.3%.In the current study, all the 30 cases (100%) presented with a breast lump, of which, 3 cases (10%) presented with associated nipple retraction, 2 cases (6.7%) with associated nipple discharge and one case (3.3%) presented with lump associated with pain. In the study conducted by Raina V et al.29, 96.5% of cases presented with a breast lump, 15.8% had pain, 9% had nipple retraction and 4.9% had nipple discharge. Pervin et al. 30observed that 100% of patients presented with a breast lump, of which 74% had pain, 20% had nipple discharge and 14% had nipple retraction. The findings of our study are more similar to that of Raina et al.[29].In the study conducted by Ambroise et al.[28]. and Azizum – Nisa et al.[31]. the left breast was more commonly involved accounting for 59.2% and 57 % respectively. In the present study, an equal number of cases (15 cases, 50%) were seen involving the right and left breast.

In the present study, most of the tumours ranged between 2-5cm, which correlated with the studies done by Ayadi L et al.[35]. and Muddawa et al. [22].The second commonest size was found to be < 2cm which correlated with Muddawa et al. [22]. The least number of cases showed tumour size of > 5cm which correlated with Ayadi et al. [35].

27 cases (90%) were Infiltrating ductal carcinoma (NOS) was the commonest histological type with 27 cases (90%) correlating with the other studies like that of Zafrani et al. [21].and Satti MB et al. [36]. In the present study, 21 cases (70%) were of histological grade 2, followed by 7 cases (23.3%) of grade 1 and 2 cases (6.7%) of grade 3 of the modified Scarff Bloom Richardson grading system. Similar observations were made by P Querzoliet al.25.

Kowalski et al.26 had 23% of tumours of grade 3 and 20%of tumours of grade. In the current study, 13 cases (43.3%), showed nodal metastasis, 12 (40%) were negative for tumour deposits while in 5 (16.7%) cases, nodes were not available for examination. Kowalski et al.[26]. found majority of lymph node status as positive (72%) while Onitilo AA et al.[37].

Found majority of lymph nodes status as negative (61.2%).

Table-10:Comparison of E-cadherin expression in breast carcinoma with other studies

E Cadherin grade	Authors		
	Kanithilatha Pai et al. [23] (n=28) (%)	P Querzoli et al.[25] (n=204) (%)	Present study (n=30) (%)
0	0	45.1	3.3
+1	7.1	31.4	26.7
+2	7.1	18.1	23.3
+3	85.8	5.4	46.7

In the current study, 46.7% of cases showed +3 grading, which does not correlate with other studies[23,25] which showed 85.8% and 5.4% of carcinoma breast cases with +3 respectively. A study that was done by Kanithilatha et al.[25] had a sample size of 28, which is closer to our study but all of which were IDC, which probably explains why none of their cases showed grade 0 E-cadherin. But there is a huge difference in the percentage of cases showing +3 between their study and the current study and thus no correlation (Table – 10).

Table–11: Correlation of E-cadherin expression with histological grade of the tumor

Histological Grade	Authors					
	Sekar et al.38 (% of E-cadherin expression)		p-value	The present study (% of E-cadherin expression)		p-value
Preserved	Aberrant	0.4	Preserved	Aberrant	0.27	
1	50		50	28.6		71.4
2	46.7		53.3	57.1		42.9
3	16.7		83.3	0		100

In our study, histological grade 3, showed 100% aberrant expression of E-cadherin which is comparable with Sekar et al. [38]. followed by histological grade 1 with 71.4% and grade 2 with

42.9% of aberrant expression of E-cadherin. Hence E-cadherin expression of histological grade 1 and 2 does not correlate with Sekar et al.[38] (Table – 11).

Table-12: Correlation between histological type and E-cadherinexpression

Histological type	Kanithilatha et al.[23] Preserved expression (%)	Sauer et al.[39] Preserved expression (%)	Kowalski et al. [26] Preserved expression (%)	Present study Preserved expression (%)
IDC NOS	85.7	15	55	48.3
ILC	0	0	0	0

Correlation between E-cadherin expression and histological type: In our study, 48.3% of IDC NOS showed preserved expression of E-cadherin which is comparable with the study of Kowalski et al.[26] that showed 55% preserved expression.

While Kanithilatha et al.[23] found that 85.7% of IDC NOS showed preserved expression, Sauer et al.[39] found that only 15% showed preserved expression, neither of which is correlating with our study. However, 0% of ILC in our study shows preserved E-cadherin expression, which is comparable with all other studies (Table – 12).

Hence in our study, we found that there was no significant correlation of E-cadherin expression with the histological type (p = 0.19). In our study, we found that there was no significant correlation of the E- Cadherin expression with the size of the tumour (p = 0.4) as 48% of tumours with tumour size ≥ 2 cm showed aberrant expression while it was preserved in 52%. Horne et al.[40] demonstrated that, 55% of tumours with tumour size ≥ 2cm showed aberrant expression vs. 45% with preserved E-cadherin expression. Sekar et al.[39]. observed, that 70% of tumours with size ≥ 2cm showed aberrant expression while only 30% showed preserved expression, which is not comparable to our study.

Conclusion

Our study was an attempt to understand the E-cadherin expression, its ability to distinguish between infiltrating ductal and lobular breast carcinomas, and its correlation with clinicopathological parameters. No correlation has been found with E-cadherin expression and histological type of carcinoma, or histopathological grade, necrosis, calcification, lymphovascular invasion and lymph node status. In contrary to the previous studies[24] which established that aberrant E-cadherin expression skews the diagnosis towards invasive lobular carcinoma rather than invasive ductal carcinoma of the breast, our study revealed 51.7% of IDC cases with aberrant expression poses a question as to how reliable a marker, E-cadherin is, in differentiating ductal and lobular invasive carcinomas in breast, hence it may not have significance in routine use.

What does this study add to existing knowledge?

Differentiation between infiltrating lobular and ductal carcinomas cannot be made by using E-Cadherin alone. There is a necessity to use newer markers to make this distinction. Recent research has shown the switch of E-cadherin to N-cadherin expression in invasive ductal carcinomas. Further studies on these emerging markers have to be done as there is a potential for newer cancer therapies.

Authors Contribution

Dr. Shruthi Thota: Data collection, data compiling, literature review, manuscript preparation, manuscript editing, final approval.

Dr. Naresh Jaikumar Kulkarni: Data collection, manuscript editing

Dr. Ramesh Chavan: Literature review, data compiling

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