

## Role of P-Cadherin as Myoepithelial Cell Marker in Differentiating Benign and Malignant Breast Lesions

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### Abstract:

**Background:** P-cadherin is cell–cell adhesion glycoprotein which can be used as a myoepithelial cell marker in the breast lesions. Myoepithelial cell layer is retained in most benign lesions and loss of this outer layer is hallmark of malignant lesions of the breast.

**Aim:** To study the usefulness of P-cadherin as myoepithelial cell marker in differential diagnosis of Benign and Malignant Breast lesions. To study the P-cadherin expression in different types and grades of invasive breast carcinomas.

**Materials And Methods:** A total of 50 cases of Benign and Malignant Breast lesions were collected from Upgraded Department of Pathology, Osmania General Hospital from September 2013 to November 2015. Immunohistochemistry using P-cadherin was done in all Benign and Malignant Breast lesions.

**Results:** Total 50 cases of breast lesions were studied,30 cases of benign and 20 cases of malignant Breast lesions. Out of 30 cases of benign breast lesions, all cases showed positivity of P cadherin immunostaining. Few cases of the malignant breast lesions showed positivity for P cadherin immunostaining.

**Conclusion:** P-cadherin is a highly sensitive marker for Myoepithelial cells and should be considered as a useful marker in the differential diagnosis of breast lesions wherever there is confusion in diagnosis with routine methods. This is evidenced by the fact that P-cadherin immunoreactivity was seen in 100% of benign cases, whereas only 22% of malignant cases were P-cadherin immunoreactive.

**Keywords:** Breast lesions, Immunohistochemistry, P-cadherin.

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### I. Introduction

Cadherins are a family of transmembrane glycoproteins involved in calcium-dependent cell–cell adhesion in many tissues<sup>(1,2)</sup>. Although these proteins are similar in their domain structure, calcium and protease sensitivity, and molecular weight, they have distinct tissue expression patterns and immunological reactivity. P-cadherin is localized in placenta, whereas E-cadherin and N-cadherin are found in epithelial and neural tissues, respectively<sup>(3-5)</sup>.

Normal breast ducts and lobules are comprised of two epithelial layers. Outer MECs are spindle-shaped contractile, smooth muscle like cells<sup>(6)</sup>. Loss of this layer is hallmark of infiltrating carcinomas in breast as it is retained in most benign as well as in ductal carcinoma in situ (DCIS)<sup>(7)</sup>. This study was conducted to assess the ability of immunohistochemical (IHC) marker, P-cadherin, to distinguish between benign and malignant breast lesions, through evaluating the P-cadherin expression in different benign and malignant lesions of breast.

### II. Materials And Methods

This study was done in the Upgraded Department of Pathology at Osmania General Hospital, Hyderabad from September 2013 to November 2015. Clinical data was retrieved from records. The specimens were fixed in 10% buffered formalin. Specimens grossed and sections were taken from representative sites. The sections were then processed in automated tissue processor (leica) and embedded in paraffin wax. A total number of 50 cases are studied comprising of 30 benign and 20 malignant cases. Normal breast tissue adjacent to pathological area was taken as control.

#### Immunohistochemistry Method:

Paraffin blocks were sectioned at 4 micron in thickness, mounted on freshly prepared 0.01% poly-l-lysine-coated slides. Slide were dried overnight at 37°C, de waxed in xylene, and hydrated. Endogenous peroxidase activity was blocked by adding freshly prepared 0.3% H<sub>2</sub>O<sub>2</sub> in methanol for 10 min followed by three washings in Tris-buffered saline (TBS). Heat-induced antigen retrieval was used with pressure cooking for 2 min in 0.1 M citrate buffer (pH 6.0). They were washed with running water and then rinsed with TBS. Sections were incubated for 30 min at room temperature with primary antibodies, monoclonal mouse anti-P-

cadherin clone 56C1. After washing thoroughly with TBS, the sections were incubated with secondary antibody for 1 h at room temperature. Sections were again thoroughly washed with TBS and incubated with tertiary antibody for another 1 h at room temperature, followed by rinsing with TBS. A drop of diaminobenzidine was then spread over the sections for 7 min and then it was rinsed in water. The sections were counter-stained with hematoxylin for 30-45 s before rinsing with running water for 3 min and dehydrated in increasing alcohol concentration and mounted.

### Immunohistochemical Analysis

Positive control section was normal breast tissue adjacent to pathological area.

Negative control section was processed similarly by omission of primary antibody.

### Interpretation Of P-Cadherin Reactivity

The P-cadherin reactivity was graded by determining the percentage of P-cadherin immunoreactive cells i.e., brown cytoplasmic reactivity and intensity of staining.<sup>(8)</sup>

Intensity scores (IS) of IHC reaction, as viewed under light microscope, are as follows:

- 0 - Negative
- 1 - Weak
- 2 - Medium
- 3 - High

Proportion score (PS) cells showing staining:

- 0 - No staining
- 1 - 1-10%
- 2 - 11-50%
- 3 - >50%

Total score (TS) = IS \*PS

Negative TS <3, Positive TS >3

### III. Results

This study included 50 cases of breast lesions comprising 30 benign breast lesions and 20 malignant breast lesions. Out of 30 cases of benign breast lesions, all cases showed positivity of P-cadherin immunostaining. Out of these cases, staining index for majority (76%) was between 4 and 6. The subgroup with staining index between 7 and 9 comprised 23% of cases. Majority of the cases with

fibroadenoma (7 in number) showed staining index of 4-6. Rest of cases of fibroadenoma (3 in number) showed staining index of 7-9. This variation is more so due to variation in intensity of staining. In 4 cases of Fibrocystic disease, 2 cases showed staining index of 4-6 and other two cases showed staining index of 7-9. In 4 cases of Ductal hyperplasia, 2 cases showed staining index of 4-6 and other two cases showed staining index of 7-9. All cases of Papilloma, Phyllodes tumor and Fibroadenosis showed staining index of 4-6.

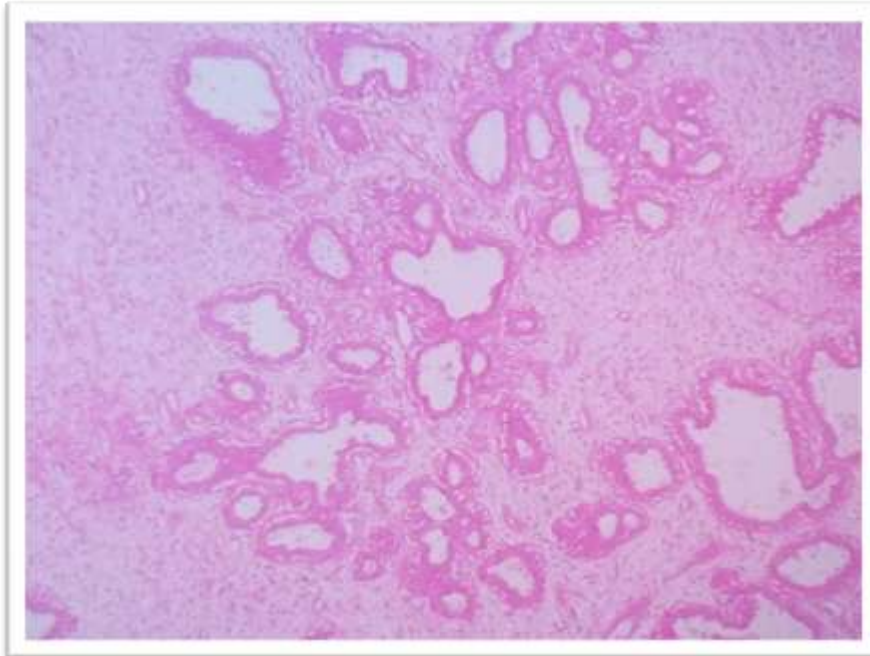
Only few cases of the malignant breast lesions showed positivity for P-cadherin immunostaining. 2 cases of DCIS showed staining index of 4-6. In 10 cases of IDCC-NOS, 2 cases showed staining index of 4-6. High grade carcinomas like Medullary carcinoma and Metaplastic carcinoma showed staining index of 4-6. None of the case had a staining index in the range of 7-9.

#### P-Cadherin immunohistochemical staining in Benign Breast lesions

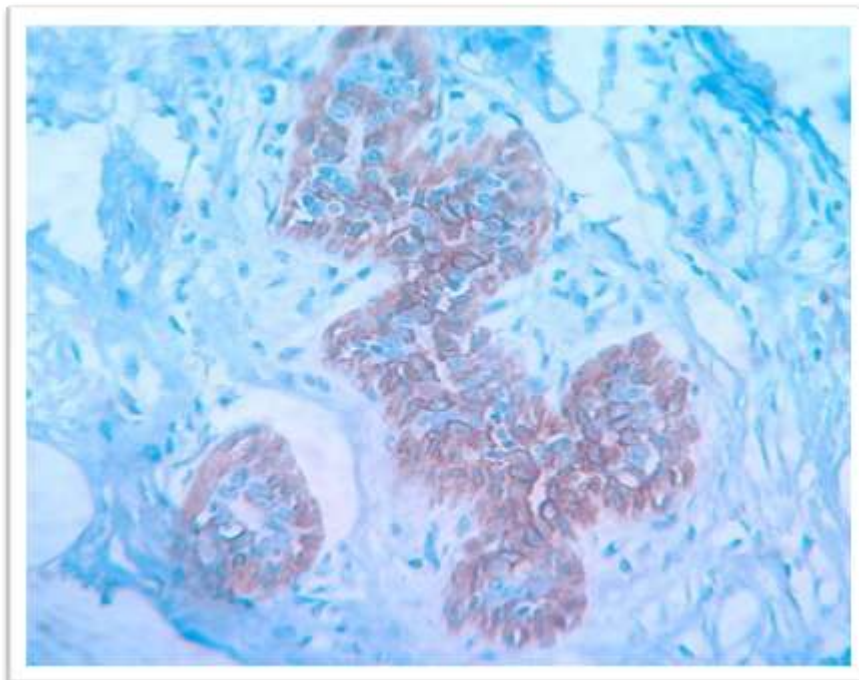
Histological diagnosis	NO of cases	Staining index			
		0	1-3	4-6	7-9
Fibroadenoma	10	0	0	7	3
Fibrocystic disease	4	0	0	2	2
Ductal hyperplasia	4	0	0	2	2
Papilloma	4	0	0	4	0
Phyllodes tumor	4	0	0	4	0
Fibroadenosis	4	0	0	4	0
Total	30	0	0	23	7

**P-Cadherin Immunohistochemical expression in Malignant Breast lesions**

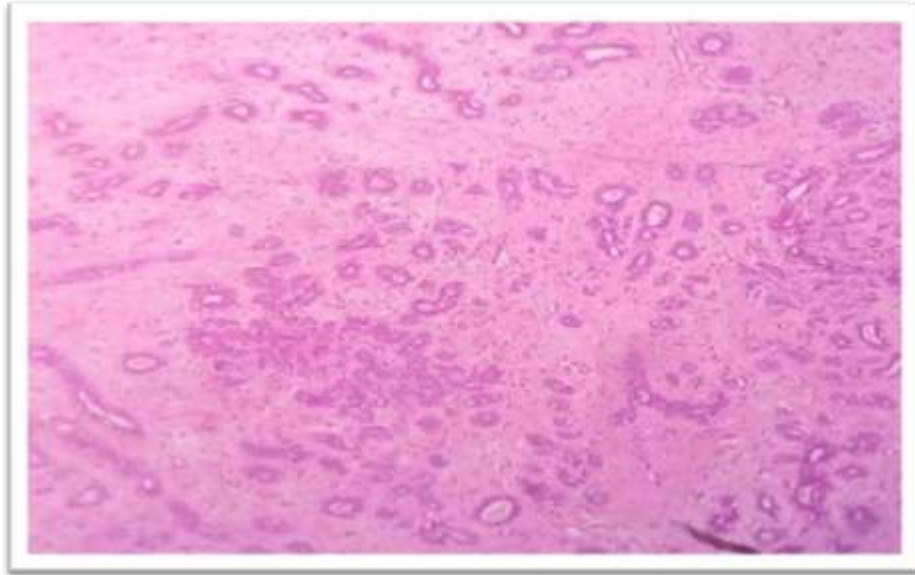
Histological diagnosis	No of cases	Staining index			
		0	1-3	4-6	7-9
DCIS	2	0	0	2	0
IDCC – NOS	10	8	0	2	0
Invasive Lobular carcinoma	3	3	0	0	0
Tubular carcinoma	3	3	0	0	0
Medullary carcinoma	1	0	0	1	0
Metaplastic carcinoma	1	0	0	1	0
Total	20	14	0	6	0



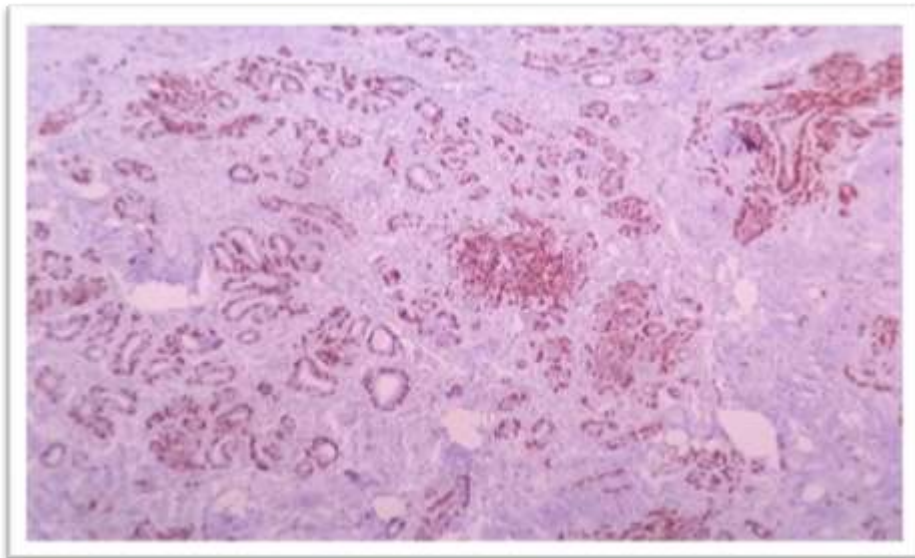
**Fibroadenoma H&E 10X**



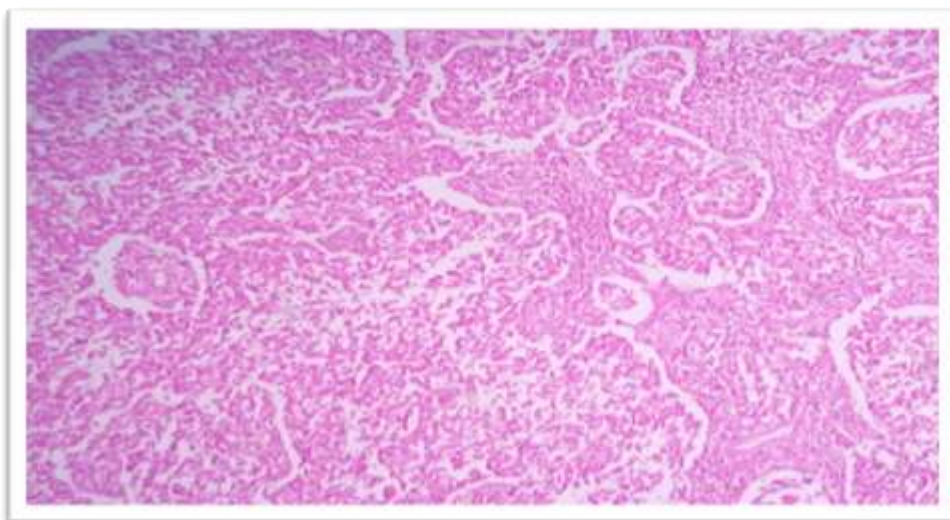
**P-cadherin expression in Fibroadenoma IHC 40X**



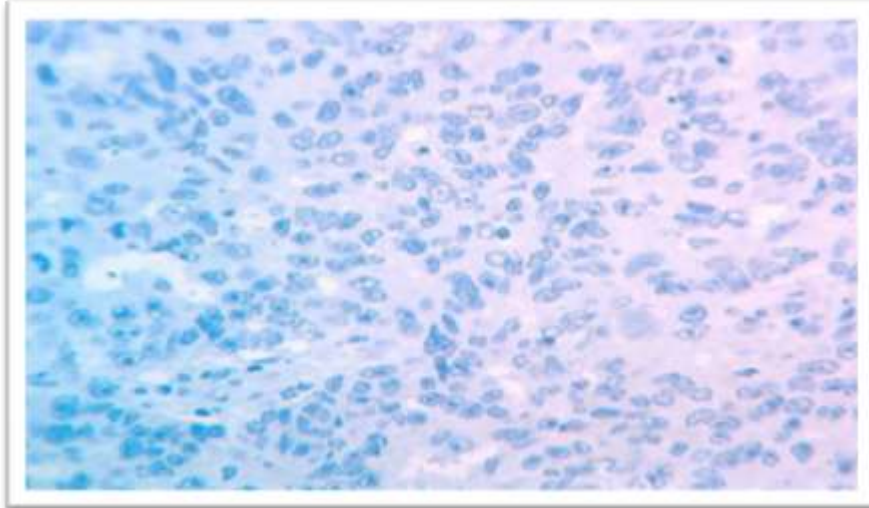
**Fibroadenosis H&E 10X**



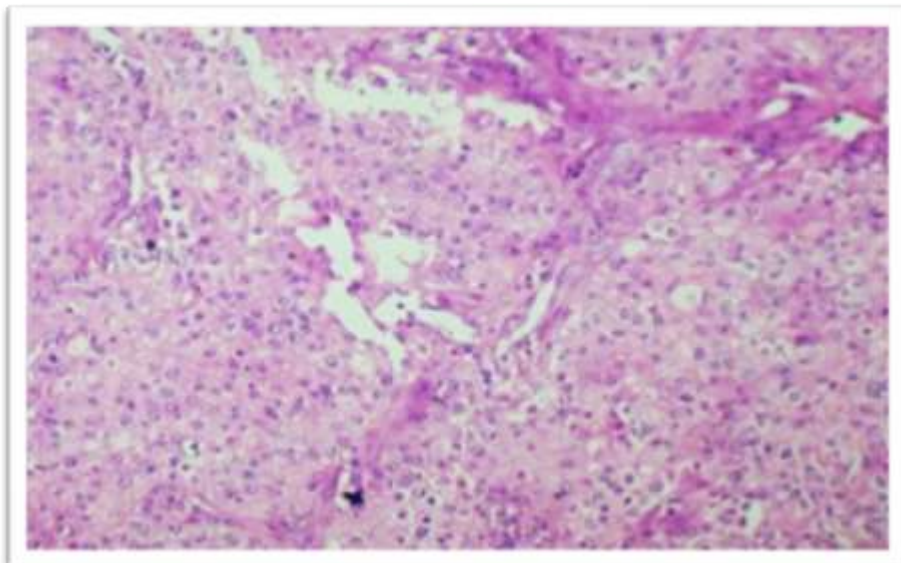
**Fibroadenosis IHC 10X**



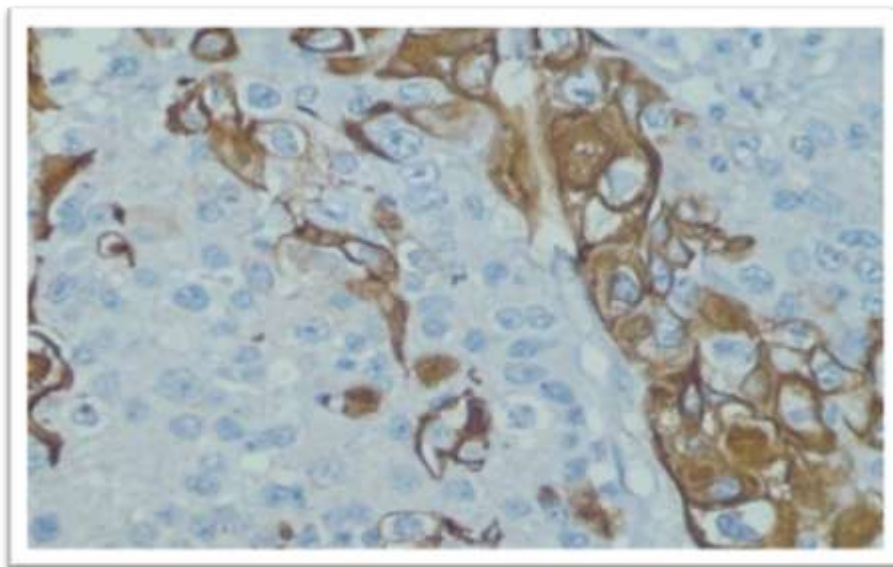
**Infiltrating ductal carcinoma H&E 10X**



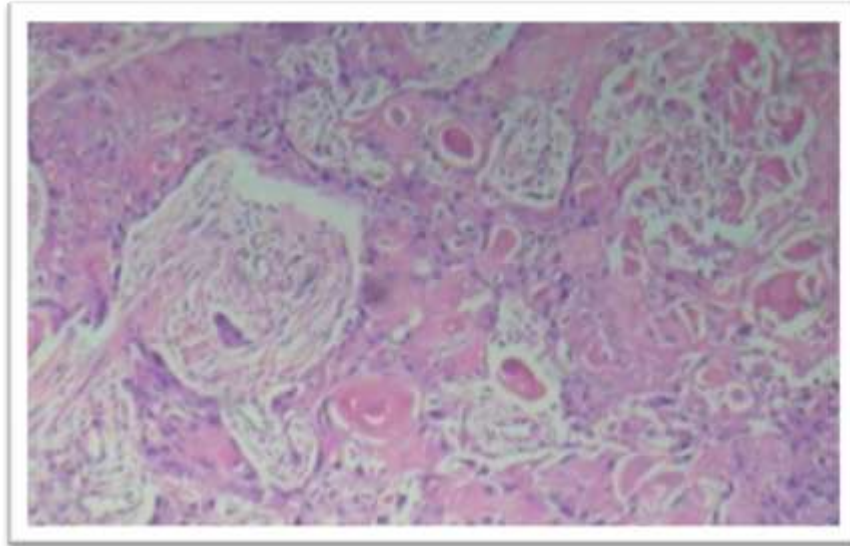
**P-cadherin expression in IDC - NOS IHC 40X**



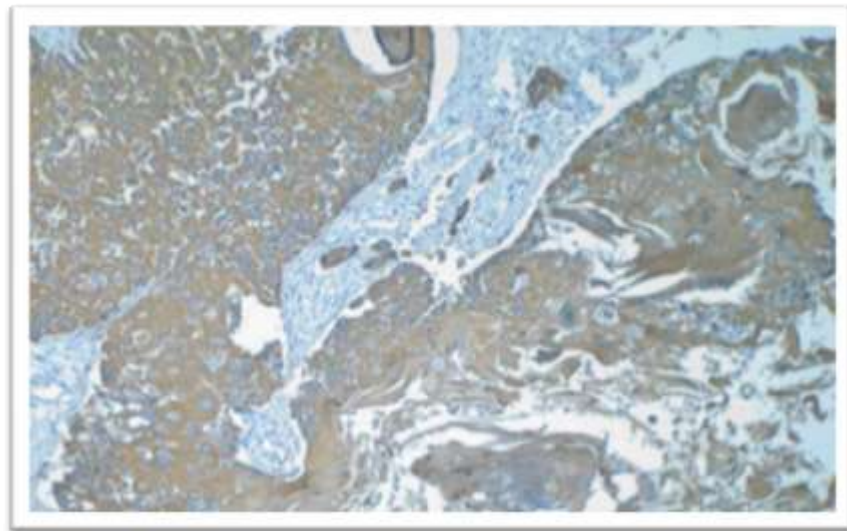
**Medullary carcinoma of Breast H&E 10X**



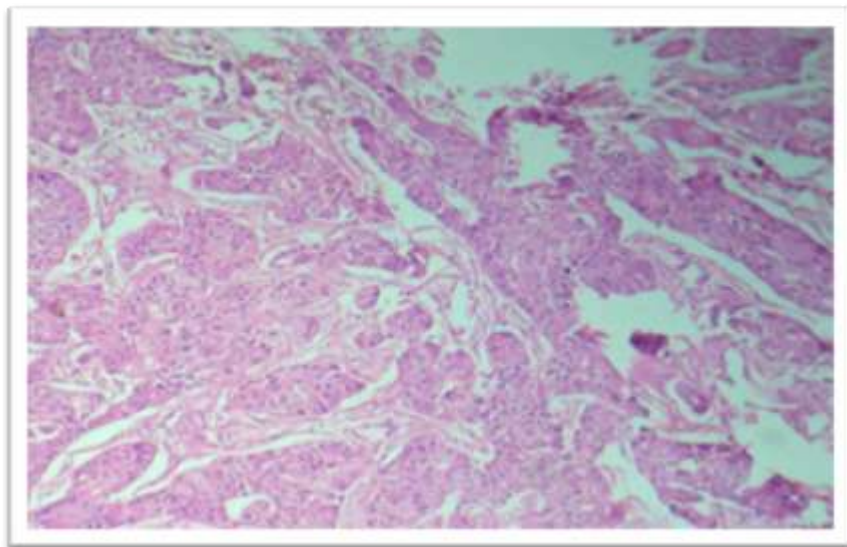
**P-cadherin expression in Medullary carcinoma IHC 40X**



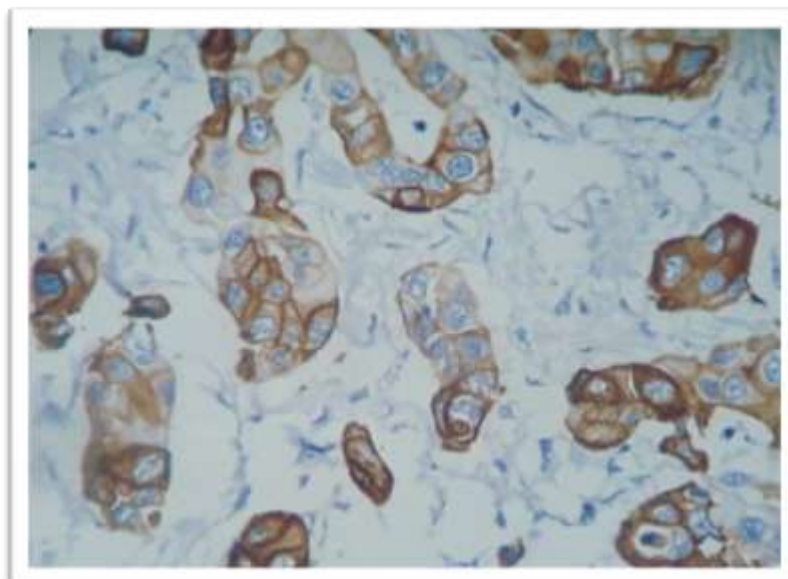
**Metaplastic carcinoma of Breast H&E 10X**



**P-cadherin expression in Metaplastic carcinoma IHC 10X**



**High grade Invasive duct cell carcinoma H&E 10X**



**P-cadherin expression in High grade IDCC IHC 40X**

#### **IV. Discussion**

Normal breast ducts and lobules are bilayered, inner luminal epithelial layer and outer myoepithelial cell layer. E-cadherin is present in both epithelial and myoepithelial cells, whereas P-cadherin is confined to myoepithelial cells. Outer myoepithelial cells are spindle shaped, contractile smooth muscle like cells. Loss of myoepithelial cell layer is the hallmark of infiltrating carcinomas in breast, as it is retained in most benign as well as in ductal carcinoma in situ. P-cadherin was identified in all Myoepithelial cells of all breast tissues studied, with no differences between ducts and lobules, with overall strong staining.

In this study the expression of P-cadherin was compared with benign and malignant breast lesions. Out of 30 Benign breast lesions in the present study involvement of right breast was 10(33.3%), left side 16 (53.3%) and bilateral 4(13.3%) cases. Study conducted by M. Kumar et al<sup>(9)</sup> of total 380 benign breast diseases showed right sided breast involvement was more common constituting 181 (47.63%) cases while left breast involvement was less common constituting 151 (39.73%) of the patients. Bilateral involvement was seen in only 48 (12.63%) of patients. In the present study the side involvement of Malignant lesions was right side 14(70%) and left side 6(30%) cases, Bhalla et al<sup>(10)</sup> observed right side (44%) and left side (56%).

In Malignant breast lesions of present study, the age distribution of Ductal carcinoma in situ and Infiltrating duct cell carcinoma was 32-55 years and 44-70 years respectively, whereas Bhalla et al observed age group of DCIS was 40-50 years and IDCC - NOS was 23-75 years. In this study the tumor size of malignant cases varied from 3cms to 8cms. Similar Findings was observed by Bhalla et al. According to Mudduwa et al<sup>(11)</sup> tumor size ranged from 0.2-14.2cms and Amin et al<sup>(12)</sup> observed tumor size of Malignant cases was 1.5- 12 cms. In this study the expression of P-cadherin was compared with benign and malignant breast lesions. P-cadherin was identified in all MECs of all breast tissues studied, with no differences between ducts and lobules, with overall strong staining. This study shows that P-cadherin expression can help in the differentiation between benign and malignant lesions. This is evidenced by the fact that P-cadherin immunoreactivity was seen in 100% of the benign breast lesions and only 22% of the malignant lesions submitted in this study. This may be attributed to the fact that P-cadherin is cell-cell adhesion protein showing expression in MEC layer, so it shows positive results in benign lesions. Yachika et al 2013<sup>(13)</sup> studied expression of P-cadherin on Benign and Malignant breast lesions and observed that P-cadherin immunoreactivity was seen in 100% of the benign breast lesions and only 27% of the malignant lesions.

P-cadherin is cell-cell adhesion protein showing expression in Myoepithelial cell layer, so it shows positive results in benign lesions. Similar findings were reported by Palacios *et al*<sup>(14)</sup> in 2002. They studied anomalous expression of P-cadherin in breast lesions, especially carcinomas. It showed that P-cadherin was detected in 95.35% cases of normal breast and benign lesions. A study conducted in 2003 by Kovács and Walker<sup>(15)</sup> also assessed the value of P-cadherin as myoepithelial marker in differential diagnosis of benign and malignant lesions of breast. All Myoepithelial cells in normal breast ducts, ductules, and lobules and sclerotic lesions showed strong staining for P-cadherin. P-cadherin was detected in the Myoepithelial cells of ducts and lobules of all 10 samples from reduction mammoplasties. There was no difference in reactivity between large and small ducts or lobules. There were similar findings in normal breast tissue associated with the lesions. These findings were similar to findings associated with this study.

In the present study P-Cadherin was expressed in myoepithelial cells only whereas according to Knudsen KA 2005<sup>(16)</sup> on cadherins and mammary gland, P-cadherin was expressed both in epithelial and Myoepithelial cells of normal breast tissue as well as benign lesions. Its expression was lost in carcinomas. In the present study, out of 10 cases of fibroadenoma 70% cases have staining index of 4-6 and rest of cases, 30% has staining index of 7-9. Most of cases showed cytoplasmic as well as membranous staining in >50% of tissue section, whereas Yachika et al observed that 75% of fibroadenoma cases have staining index of 4-6 and rest of them 25% has staining index of 7-9. Out of 20 malignant cases, 14 cases 70% showed no staining index (staining index was zero) and six cases 30% were immunopositive for P-cadherin with all cases having staining index of 4-6. Yachika et al studied 15 malignant cases in that 8 cases showed no staining index, four cases have staining index of 4-6, rest of three cases showed staining index of 1-3. Palacios et al. in 2002. studied anomalous expression of P-cadherin in breast carcinoma. P-cadherin expression was detected in 9 out of 45 cases (20%) of infiltrating ductal carcinomas not otherwise specified.

High grade carcinomas like Medullary carcinoma and Metaplastic carcinomas of breast are observed immunoreactively positive for P-cadherin in this study. According to Paredes et al majority of P-cadherin positive tumors were Invasive ductal carcinomas, Metaplastic and Medullary carcinomas of breast. Hans et al 1999<sup>(17)</sup> reported P-cadherin expression in almost all studied cases of Medullary, carcinosarcomas and sarcomatoid Metaplastic breast carcinomas. In this study Out of 20 malignant cases, enlarged lymphnodes are 68, in that 39 lymphnodes shown metastatic deposits and remaining 29 cases showed reactive hyperplasia. Figueira et al<sup>(18)</sup> studied 60 cases of Malignant breast lesions, enlarged lymphnodes are 35, in that 17 lymphnodes are reactive and 18 lymphnodes showed metastatic deposits. This study demonstrated an association between expression of P-cadherin and malignant phenotype, higher histological grade and invasive behavior, suggesting that this protein is a reliable independent biomarker of poor prognosis in Breast cancers. P-cadherin was identified in all myoepithelial cells of all breast tissues studied, with no differences between ducts and lobules, and overall strong staining. This consistency of reactivity is better than cytokeratins, which may have a high specificity for myoepithelial cells, but a low sensitivity, with not all myoepithelial cells reacting. Calponin has a high sensitivity, but myofibroblasts may stain, which can be a problem when assessing invasion.

P-cadherin is a highly sensitive marker for myoepithelial cells, with exceptions in few cases of benign and malignant proliferations. The distinct staining of myoepithelial cells for P-cadherin and the lack of staining for myofibroblasts, when differentiating between a radial scar and a tubular carcinoma, show its advantage over smooth muscle actin. Thus, P-cadherin should be considered as a helpful tool in the differential diagnosis of breast lesions.

## V. Conclusion

This study shows that P-cadherin expression strongly correlates with the type of breast lesion, which can help in the differentiation between benign and malignant lesions whenever there is confusion on routine staining. This is evidenced by the fact that P-cadherin immunoreactivity was seen in 100% of benign cases, whereas only 22% of malignant cases were P-cadherin immunoreactive. Thus, P-cadherin is a highly sensitive marker for Myoepithelial cells and should be considered as a useful marker in the differential diagnosis of breast lesions. P-cadherin positive expression in high grade carcinomas is considered as good indicator of poor prognosis.

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