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Original research article

Analysis of the role of E-cadherin and cytokeratin 5/6 in sub classification of breast cancer

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Abstract

Different cytokeratins are expressed in different epithelial types and at different stages of differentiation. Consequently, different epithelial types have different specific cytokeratin expression profiles, which usually remain constant after neoplastic transformation. E-cadherin is a transmembrane glycoprotein that mediates a cell–cell adhesion in the epithelial tissues. The intracytoplasmic domain of E-cadherin binds to the actin cytoskeleton through interactions with catenin proteins p120, b- catenin, a-catenin, and d-catenin in the cytoplasm. The study material included all the breast specimens (n=180) received as modified radical mastectomy with exclusion criteria of post radiotherapy and post chemotherapy in histologically proven cases of carcinoma of the breast. IHC with CK 5/6 was done in six cases, of which three cases are with DCIS component, one case of intraductal papillary ca, one case of lobular carcinoma, one case of tubular carcinoma.

Keywords: E-cadherin, Cytokeratin 5/6, breast cancer

Introduction

Breast cancer is the most common malignancy in women around the world. It is estimated that worldwide over 508 000 women died in 2011 due to breast cancer. There were about 14.9 million new cases in the world in 2012. Breast cancer has a high incidence rate in all countries. It includes 1.7 million new cases per year and 25% of all types of cancers, and is the second most common cancer in the world. The incidence rate of breast cancer ranges from 19.4 per 100,000 people in East Africa to 89.7 per 100,000 in West Europe. In most of the developing regions, the incidence rates are below 40 per 100,000. Although breast cancer is thought to be a disease of the developed world, almost 50% of breast cancer cases and 58% of deaths occur in under developed countries [1, 2].

There is at least a 10-fold variation in breast cancer incidence rates worldwide, largely due to socio-economically correlated differences, prevalence of divergent reproductive, hormonal and nutritional factors among different populations. In some high-resource countries, mammographic screening has considerably affected breast cancer diagnosis, registration, and mortality. As a consequence of changing exposures to reproductive and nutrition-related determinants over time, women are at increasingly high risk of breast cancer, with incidence rates increasing in the past few decades in most countries and regions of the world. The most rapid rises are seen in developing countries, where breast cancer risk has historically been low relative to industrialized countries. Increasing trends in developing areas are often considered the result of the westernization of lifestyles, changes in factors such as childbearing, dietary habits and exposure to exogenous oestrogen, towards a distribution closer in profile to that of women in industrialized countries [3, 4].

Cytokeratins are the fingerprinting of carcinomas in general. Cytokeratins are intermediate filament proteins, reflect the epithelial cell type, state of tissue growth, differentiation, functional status. Cytokeratins, a complex family composed of more than 20 isotypes are divided into two types. Type I (acidic group) include cytokeratins 9–20, and Type II (basic group) include cytokeratins 1-8 [5].

Different cytokeratins are expressed in different epithelial types and at different stages of differentiation. Consequently, different epithelial types have different specific cytokeratin expression profiles, which usually remain constant after neoplastic transformation.

E-cadherin is a transmembrane glycoprotein that mediates a cell–cell adhesion in the epithelial tissues. The intracytoplasmic domain of E-cadherin binds to the actin cytoskeleton through interactions with catenin proteins p 120, b- catenin, a-catenin, and d-catenin in the cytoplasm. Various molecular mechanisms inactivate or down-regulate E-cadherin and lead to disruption of cadherin-catenin complexes between cells, resulting in the loss of cellular cohesion characteristic of lobular lesions. Most

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commonly, E-cadherin is inactivated via deletions, mutations, or promoter methylation of the CDH1 gene. E-cadherin is also a prognostic marker for various carcinoma types such as breast and transitional carcinoma as the loss of E-cadherin expression is associated with aggressive behaviour. Complete loss of the E-cadherin expression occurs in most invasive lobular carcinomas and lobular carcinomas in situ, but not in invasive ductal cancers or ductal carcinomas in situ [6].

From a management standpoint, E-cadherin is important in distinguishing classic LCIS from DCIS. The aim of treating DCIS is local eradication by surgical excision with negative margins, usually followed by adjuvant radiotherapy. Treatment of classic LCIS, in contrast, is less aggressive and usually includes clinical/imaging follow-up, with or without adjuvant endocrine therapy.

Methodology

The study has been conducted in the Department of Pathology, in cooperation with the Department of General Surgery.

The study material included all the breast specimens (n=180) received as modified radical mastectomy with exclusion criteria of post radiotherapy and post chemotherapy in histologically proven cases of carcinoma of the breast.

The technique of analysis

- Specimens were fixed in 10% formalin.
- Tumor size was assessed during grossing of the specimen, and a minimum number of 4 bits were taken from the tumor proper.
- Tumor tissue and all the lymph nodes identified were processed.
- Paraffin blocks were cut at 4 microns thick and stained with Haematoxylin and Eosin.

Classification of tumors was done according to the WHO classification of tumors of the breast. Tumor grade was assessed on H&E using the Elston & Ellis Modified Bloom & Richardson grading system.

- IHC with E-Cadherin was performed in all cases of ILC, selected cases of ICNST, cases of ICNST with foci resembling ILC and Tubular carcinoma.
- IHC with CK5/6 was performed in cases with DCIS component, ILC, Intraductal papillary carcinoma, and Tubular carcinoma.

The procedure of H&E staining

- Xylene – 20 minutes
- 70% Ethanol – 5 dips
- Distilled water – 5 dips
- Harris Haematoxylin – 2 minutes
- Washing in running tap water – 30 minutes
- Acid alcohol – 2 to 3 dips
- Washing in running tap water – 30 seconds
- Eosin – 20 seconds
- Washing in running water – 1 minute
- 70% Ethanol – 5 dips
- Absolute Ethanol – 5 dips
- Xylene
- Mounting with DPX.

Reagents used for E- Cadherin

Immunogen: Transmembrane glycoprotein Clone: EP6
Isotype: Rabbit IgG Reactivity: Human

Reagents used for Cytokeratin 5/6

Immunogen: Cytoplasmic intermediate filament protein Clone: CK5: EP24; CK6: EP67
Isotype: Rabbit IgG Reactivity: Human

Results

IHC with E-cadherin was done for 13 cases, of which 2 were ILC, 8 were ICNST, 2 were ILC/ICNST and one tubular carcinoma.

Table 1: Number of cases for IHC E-cadherin

Histological Type	Number of cases for IHC
ILC	2
ICNST	8

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ICNST/ILC	2
Tubular carcinoma	1
Total	13

Table 2: Interpretation of IHC E-cadherin

Histological type of breast cancer	E-Cadherin staining score			
	3+	2+	1+	0(negative)
ILC(n=2)	0	0	0	2
ICNST(n=8)	7	1	0	0
ICNST/PLC(=2)	1	1	0	0
Tubular carcinoma (n=1)	1	0	0	0

Note: A strong inter-membranous staining in most of the tumour cells was scored as 3+, moderate staining in >10% of the cells was scored as 2+,weak staining in < 10% cells was scored as 1+, and an absence of membrane staining was scored as 0

IHC with CK 5/6 was done in six cases, of which three cases are with DCIS component, one case of intraductal papillary ca, one case of lobular carcinoma, one case of tubular carcinoma.

Table 3: Number of cases for IHC CK5/6

Histological Type	Number of cases for IHC
With DCIS component	3
Intra ductal papillary ca	1
ILC	1
Tubular ca	1
Total	6

Table 4: Interpretation of IHC CK5/6

Cytokeratin 5/6 Expression in cases with in situ areas	CK 5/6 intensity scoring	CK 5/6 Proportionate score	Result
With DCIS	1) 3	4	(3+4=7) Positive
component (n=3)	2) 3	5	(3+5=8) Positive
	3) 3	4	(3+4=7) Positive
Intraductal papillary carcinoma (n=1)	3	5	(3+5=8) Positive
Lobular carcinoma (n=1)	0	0	(0+0) Negative
Tubular carcinoma (n=1)	0	0	(0+0) Negative

Note: The breast lesion with score < 2 were termed cytokeratin 5/6 negative while those with score >2 were termed cytokeratin 5/6 positive

IHC E-cadherin in ICNST with rows of cells

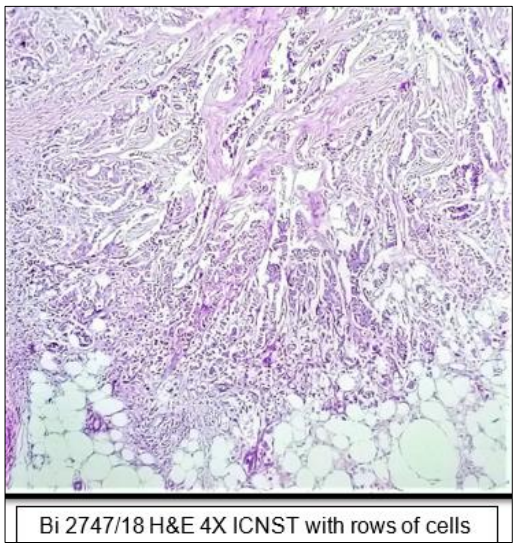

Fig 1: Photomicrograph of ICNST with foci showing small dyscohesive tumor cells arranged in rows

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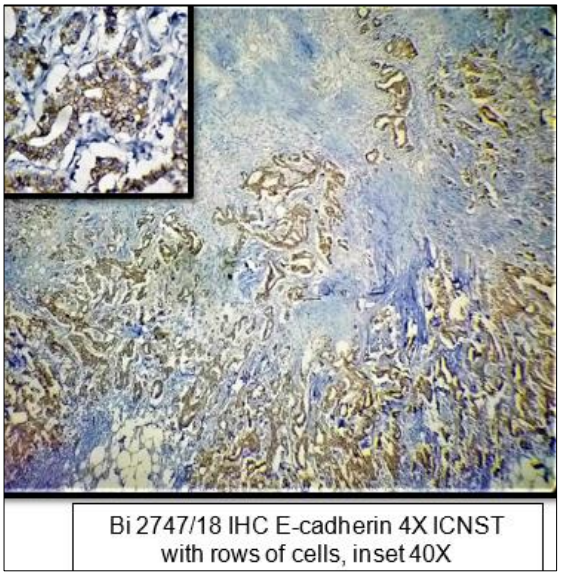

Fig 2: Photomicrograph of ICNST with rows of cells showing IHC E-cadherin 3+ positivity

IHC E-cadherin in Tubular carcinoma

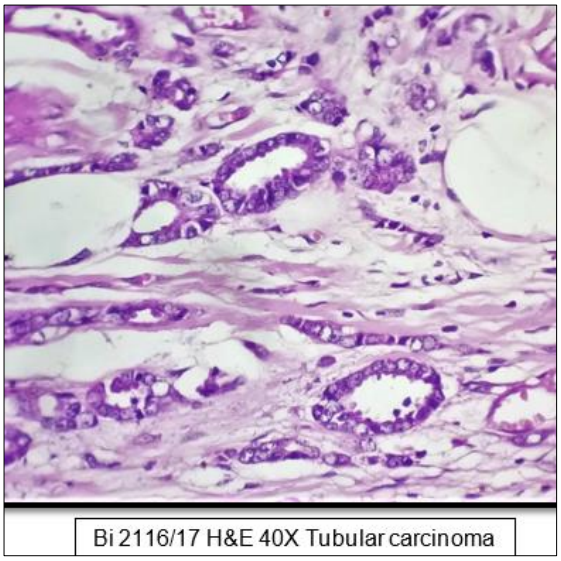

Fig 3: Photomicrograph of Tubular carcinoma showing open tubules

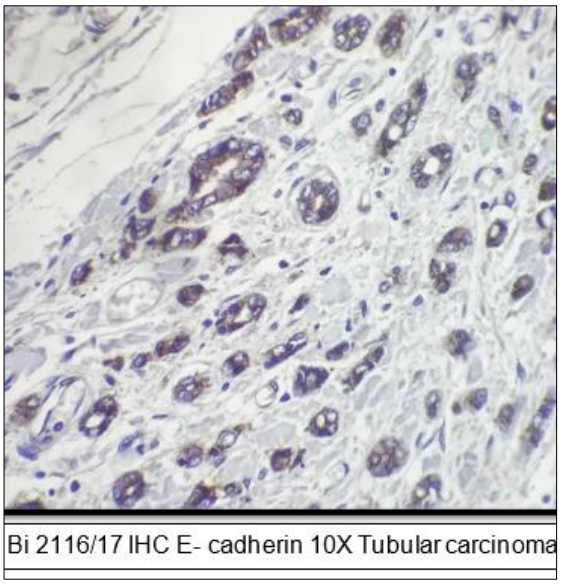

Fig 4: Photomicrograph of Tubular carcinoma showing IHC E-cadherin 3+ positivity in open tubules

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IHC E-cadherin in Tubular carcinoma

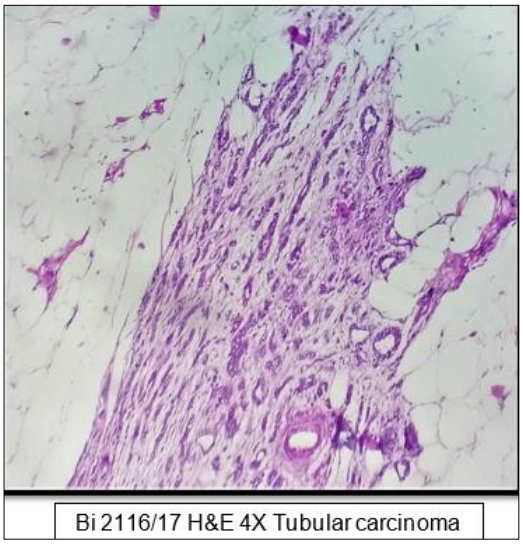

Fig 5: Photomicrograph of Tubular carcinoma showing rows of tumor cells along with open tubules

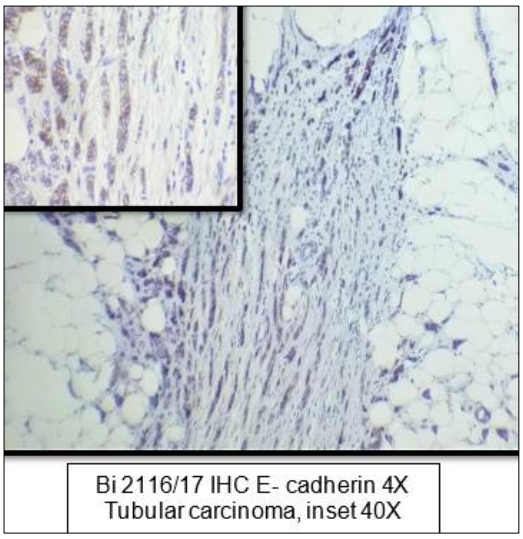

Fig 6: Photomicrograph of Tubular carcinoma showing IHC E-cadherin 3+ positivity in open tubules and rows of cells

IHC CK5/6 in DCIS

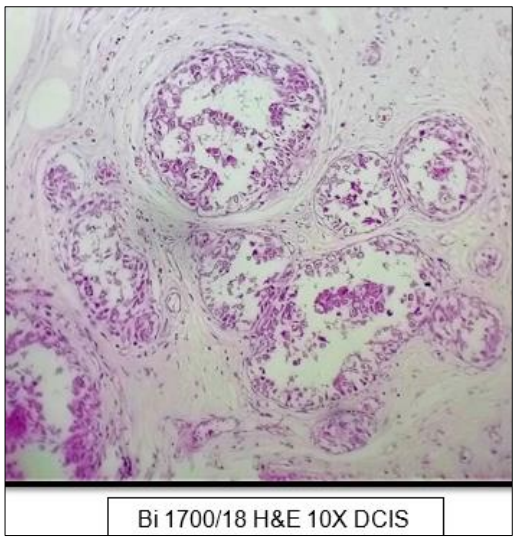

Fig 7: Photomicrograph of DCIS showing tumor cells surrounded by myoepithelial cells

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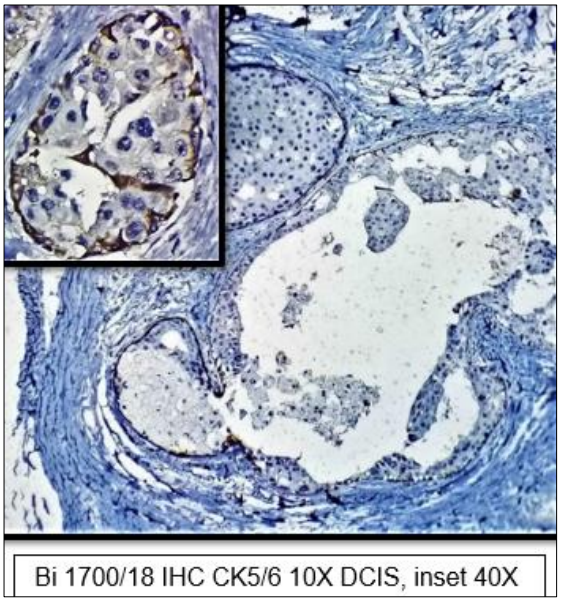

Fig 8: Photomicrograph of DCIS with IHC CK5/6 showing positive CK5/6 myoepithelial cells

IHC CK5/6 IN Intraductal papillary carcinoma

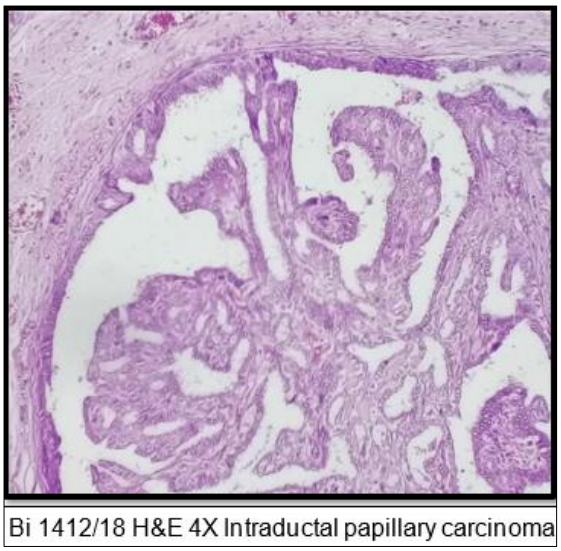

Fig 9: Photomicrograph of Intraductal papillary carcinoma showing tumor cells surrounded by myoepithelial cells

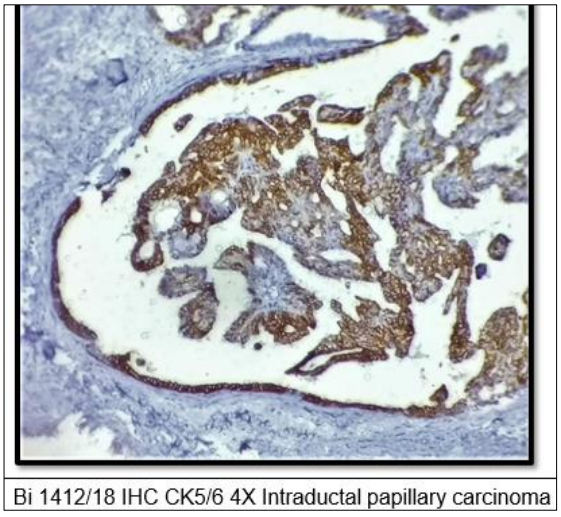

Fig 10: Photomicrograph of Intraductal papillary carcinoma with IHC CK5/6 showing positive CK5/6 myoepithelial cells

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IHC CK5/6 IN ILC

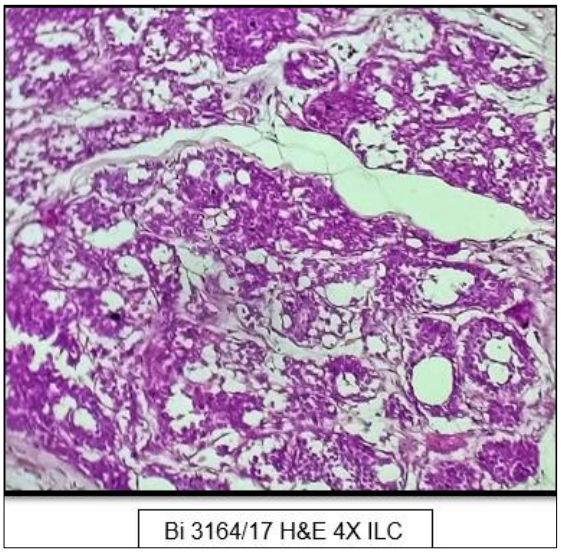

Fig 11: Photomicrograph of ILC showing small dyscohesive tumor cells

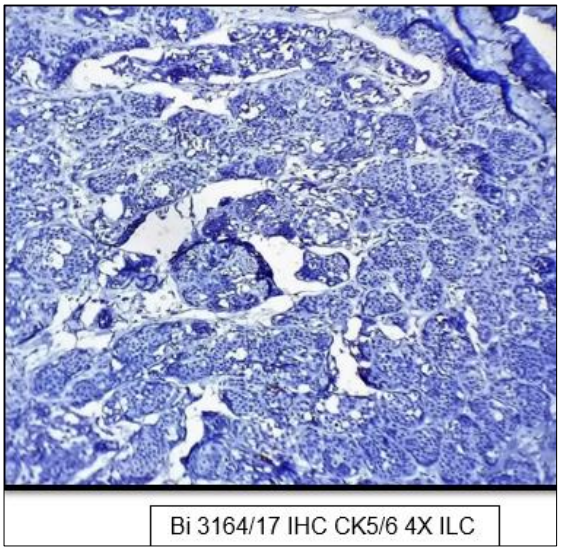

Fig 12: Photomicrograph of ILC with IHC CK5/6 showing small dyscohesive tumor cells with score 0

IHC CK5/6 IN Tubular carcinoma

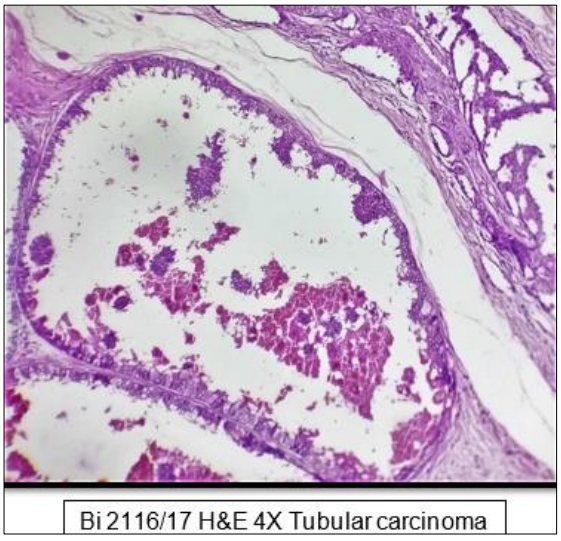

Fig 13: Photomicrograph of Tubular carcinoma showing dilated tubule with neoplastic cells

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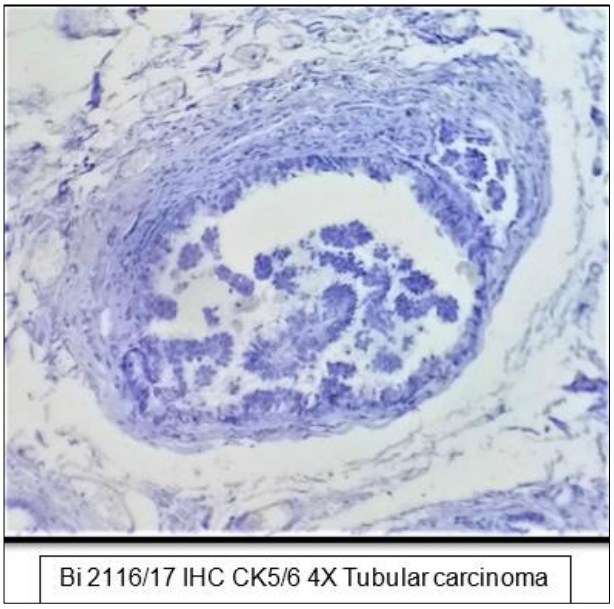

Fig 14: Photomicrograph of Tubular carcinoma with IHC CK5/6 showing dilated tubule with neoplastic cells score 0

Discussion

In a majority of cases of lobular carcinoma, characteristic morphological features of the tumour cells will clinch the issue on routine H&E sections. However diagnostic problems can arise in certain cases wherein distinguishing lobular carcinoma from low grade ICNST becomes difficult basing on morphology alone. In this scenario work up with IHC marker E- cadherin, the loss of which is a consistent feature of lobular cancer, is of great utility in confirmation of the diagnosis. E-cadherin expression in breast carcinoma is more related to histological type and differentiation grade than with invasiveness and metastatic potential.

In the present study, E-cadherin was done in 13 cases which required a Immunohistochemical workup to supplement and confirm histomorphological diagnosis. Of these, two cases were interpreted as ILC and eight cases as ICNST on H & E sections. Two cases posed diagnostic problems on H & E as foci showed single rows of cells apart from the predominant features of ICNST. Another case was diagnosed as tubular carcinoma but subjected to E-cadherin to exclude tubulo lobular carcinoma.

Of these 13 cases, E-cadherin positivity with ≥ 2+ was observed in all the eight cases of ICNST thus confirming the histopathological diagnosis. In the other two cases of ICNST with focal Indian file pattern and one case of tubular carcinoma also, E-cadherin expression was ≥ 2+ thus excluding the possibility of lobular component (picture 31, 33). Negative score with 0+ was observed in both the cases of ILC diagnosed on H&E.

These observations correlate with recorded literature according to which complete loss of the E-cadherin expression occurs in most invasive lobular carcinomas and lobular carcinomas in situ, but not in invasive ductal cancers or ductal carcinomas in situ.

Table 5: Comparison of E- cadherin expression

	Present study	**Kanthilatha *et al* [7]**	**HinaS *et al* [8]**	**Rajeev Singhai *et al* [9]**
ILC (E-cadherin negative score)	100%	82%	90	90
ILC (E-cadherin positive score)	0%	18%	10	10
ICNST (E-cadherin negative score)	0%	0%	0.5%	0.5
ICNST (E-cadherin positive score)	100%	100%	99.5%	99.5
ICNST/ILC (E-cadherin negative score)	0%	-	44	44
ICNST/ILC (E-cadherin positive score)	100%	-	56	56

The findings in the present study are similar to those of Hina S *et al.* [8] and Rajeev Singhai *et al.* study [9]. There is variation in the expression of E- cadherin in ILC when compared with Kanthilatha *et al.* [7] study which reported only 82% of negative E-cadherin staining in ILC while in the present study it was 100%. Positive E-cadherin expression in Kanthilatha *et al.* study was 18% while in present study it was 0% as shown in table 22. Some of these E cadherin positive lobular carcinomas in these studies could be ICNST as the morphological overlap between the two entities was described in the literature.

Tubular carcinoma showed positive E-cadherin staining with 3+ score in the present study which correlates with the Hina S *et al.* [8] study which reported positive E-cadherin expression in 4 cases of

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tubular carcinoma.

Cytokeratin 5/6, an intermediate filament protein, is expressed by the basal / myoepithelial cells along with few other cytokeratins (CK 14, CK 17) and smooth muscle actin. This antibody is applied very frequently to differentiate low grade invasive tumours from noninvasive lesions because the myoepithelial layer is absent in low grade invasive carcinomas, and as such CK 5/6 expression is negative.

CK 5/6 expression should be interpreted along with H & E morphology in the differential diagnosis of preinvasive breast lesions. Otterbach *et al*. [10] reported that usual ductal hyperplasia showed a large number of CK5/6 positive cells whereas in the atypical lesions no cells or only a few luminal cells showed positivity for CK 5/6. In the present study, IHC with CK 5/6 was done in six cases of carcinoma breast. Three of these cases showed predominant foci of ductal carcinoma in situ on H&E. The remaining cases comprised one case of intraductal papillary carcinoma, one lobular carcinoma and one tubular carcinoma.

The staining pattern was interpreted based on both the proportion of cells expressing the marker and the intensity of staining. In the present study, CK 5/6 expression with >2 score (scores 7 to 8) was observed in three cases with predominant ductal carcinoma in situ. CK 5/6 was evident in the cells around the tumor cells reflecting the intact myoepithelial cells surrounding the in situ carcinoma component in these cases.The present study thus showed high positivity of CK 5/6 (100%) in the foci of in situ carcinoma when compared with the study of Yang *et al*. [11] who reported 76.5% positivity.

In the case of intraductal papillary carcinoma, CK 5/6 positivity was observed with >2 (score 8) in the myoepithelial cells in the duct enclosing the intraductal papillary carcinoma.

In contrast, the cells at the invasive component of ICNST, ILC and tubules in the tubular carcinoma expressed no cytokeratin positivity confirming the absence of myoepithelial cells in the invasive component of these tumours. Negative expression of CK5/6 in invasive carcinoma in the present study (100% CK5/6 negative) correlates with the study of Yang *et al*. [11] (100% CK5/6 negative).

However, CK 5/6 expression should be interpreted carefully to avoid pitfalls of diagnosis. In DCIS with spindle cells, there may be no immunostaining with CK 5/6. On the other hand, myoepithelial carcinoma of the breast is a malignant spindle cell lesion that exhibits a positive immunostaining [12].

Conclusion

IHC with E-cadherin has been used to differentiate between ILC and ICNST in cases with equivocal features, as there is a positive correlation between the E-cadherin expression and ICNST and the loss of E-cadherin and the diagnosis of ILC and its variants. A strong, complete, membranous E- cadherin expression helps in resolving the problem and in aiding in the sub classification of invasive breast carcinoma as ICNST but not ILC.

In situ carcinoma can be differentiated from the invasive component by establishing the presence or absence of myoepithelial cells which is facilitated by study with IHC marker CK 5/6. In cases with equivocal histomorphology, positivity for CK 5/6 indicates the intact myoepithelial layer surrounding the tumour cells clinching the diagnosis of in situ carcinoma. In contrast, absence CK 5/6 expression reflects the loss of myoepithelial cells confirming the cases as invasive carcinoma.

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