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Diagnostic Value of Cytokeratin 5/6 in Benign and Malignant Breast Lesions

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Abstract

Background: The presence of epithelial layer along with myoepithelial layer is the basis of benignity for any breast lesion. This study sought to determine if the Value of cytokeratin 5/6 in benign and malignant breast lesions is diagnostic. Cytokeratins are intermediate filaments present in all epithelial cells so it's positivity in myoepithelial cells differentiate benign lesions from malignant lesions.

Material and Methods: Hospital based observational study, carried out on biopsy samples received. Tissue were fixed in formal saline, paraffine blocks were prepared and stained with Haematoxylin and Eosin (H&E). Immunohistochemical (IHC) staining for cytokeratin 5/6 was applied on paraffin embedded sections of 40 samples using avidine biotin peroxidase technique with mouse antihuman polyclonal D5/16B4 antibody and visualized with DAB. The pattern and intensity of staining of tumor cells was observed and graded.

Findings: 40 cases of breast lesions were taken, out of which 26 were benign and 14 were malignant. All 26(65%) cases of benign lesions comprising of fibroadenoma and fibrocystic disease were strongly positive. Out of 14(35%) cases 13 cases of IDC(NOS) and 1 case of medullary carcinoma breast 07 (53.8%)cases of IDC(NOS) were CK 5/6 positive with variable staining index. Benign lesions were strongly positive as compare to malignant lesions.

Conclusion: Benign lesions are strongly positive for CK5/6 but if malignant lesions are positive for CK5/6, it indicates poor outcome due to basal molecular subtype and have an aggressive behavior.

Keywords: Breast lesions; Cytokeratin 5/6; Immunohistochemical staining; Observational study; Fibroadenoma, fibrocystic disease.

Introduction

Breast cancer is the most common malignancy in women. Advances in imaging techniques and the increased use of needle biopsy have greatly assisted the preoperative evaluation of breast lesions but, in a large proportion of cases, differentiation between benign and malignant lesions still rests on histologic examination.[1] The rising prevalence of breast cancer and molecular diagnosis of the breast cancer variants continue to concern the medical community. Molecular diagnosis of the breast cancer types depends on highlighting with tumor-marker stains, the molecules considered as signals, symbols or representatives of tumor cells and which are increased in the cancerous conditions. Normal cells also express most of the tumor markers, but quantum of marker molecules distinguishes the tumor cells from the normal cells.[2]

In the majority of cases, diagnosis of benign and malignant epithelial lesions of the breast are achievable using hematoxylin and eosin (H&E)

microscopic sections alone. However, in some circumstances, such as in atypical and borderline breast lesions, as well as in core needle biopsy (CNB), this morphological distinction can be problematic. In addition, inter-observer variability exists among pathologists when interpreting difficult and borderline lesions of breast. Therefore, using immune-histochemical (IHC) stains can be of help when dealing with these challenging lesions, particularly in cases for which the diagnosis carries a significant impact on management and prognosis.[3,4] Cytokeratin(CK), an intermediate filament protein, reflects the epithelial cell type, state of tissue growth, differentiation, functional status and is used for the fingerprinting of various carcinomas.[5]

Materials and Methods

The study is carried out on biopsy samples received on breast tissue, in Histology clinic of Department of Pathology, S. N. Medical College, Agra with a clinical diagnosis of breast lump of 40 patients. Study has been carried out for one and half year duration. Current study involve, Fibroadenoma, Fibrocystic disease of breast, Infiltrating ductal carcinoma NOS and Medullary carcinoma.Tissues were fixed in formal saline, processed for paraffin sections and stained with Haematoxylin and Eosin (H&E). Poly-L-lysine hydrobromide (0.1%) coated slides were used to perform immunochemistry for CK 5/6.Primary antibody Dako FLEX Monoclonal mouse antihuman Cytokeratin 5/6 clone D5/16 B4 was used, visualization obtained with DAB (3, 3'-Diamino benzidine), Pressure cooker used for target antigen retrieval. Slides were examined for staining pattern (cytoplasmic or membranous) and intensity of staining in tumor cells.

The inclusion criteria were female patients clinically presented with breast lump and surgically resected breast tissue. Exclusion criteria were female patient less than 10 years and more than 75 years, Lactating adenoma.

Results

Total 40 cases were studied for immunostaining of cytokeratin 5/6 over histological sections of breast lesions received in Histology lab. Out of 40 cases 26 cases were benign and 14 cases were malignant. 22 were of fibroadenoma of breast, 4 cases were of fibrocystic disease, 13 cases of infiltrative ductal NOS and 1 case of medullary carcinoma of breast.

Table 1: Cytokeratin 5/6 positivity in benign breast disease.

S.No	Histological type of lesion	Total cases	Cases with positive staining	Cases with negative staining
1	Fibroadenoma	22	22	00
2	Fibrocystic diseases	4	4	00
	Total cases	26	26	00

Table 1 show that total 26 benign cases of breast was taken. All 22 (100%) cases of fibroadenoma shows positive immunostaining for cytokeratin 5/6 with staining index ranging from 6–9 and they show membranous as well as cytoplasmic staining.

All 4 (100%) cases of fibrocystic disease of breast also show positive immunostaining for CK 5/6 with strong staining index (6–9) just like fibroadenoma. Pattern of staining was similar to fibroadenoma (membranous and cytoplasmic, both). Although intensity of staining and proportion of positive staining varies among the cases but all range 6–9 of staining index.

Table 2: Cytokeratin 5/6 positivity in malignant breast disease.

S.No	Lesions	Total Cases	Cases with positive staining	Cases with negative staining
1	IDC	13	7	6
2	Medullary carcinoma	1	0	1
	Total	14	7	7

Table 2 shows that 14 cases of malignant lesions of breast. Out of 13 infiltrating ductal carcinomas 6 cases were immune negative for CK 5/6 staining. The 7 cases of IDC show positive staining for CK 5/6 but they have lower staining index than the benign lesions and most of them only shows fine granular cytoplasmic positivity except for 01 case of IDC that shows membranous positivity also. Thus total 50% cases of all malignant cases show positive immunostaining with CK 5/6 or 53.84% cases of all IDC shows positive immunostaining. And 1 case of medullary carcinoma shows negative staining for CK 5/6 immunostaining.

Table 3: Correlation between benign breast disease and cytokeratin 5/6 expression.

S. No	Lesions	No.of cases	Age (years)	CK +ve cases	Stain score range
1	Fibroadenoma	22	15–40	22	6–9
2	Fibrocystic disease	4	50–60	4	6–9

Table 3 shows that staining score for all benign lesions range from 6–9, that means strong positivity

for CK 5/6 with membranous as well as cytoplasmic in both fibroadenoma as well as fibrocystic disease.

Table 4: Correlation between malignant breast disease and cytokeratin 5/6 expression.

S. No	Lesion	No. of cases	Age (years)	CK +ve Cases	CK –ve cases	Stain score range in +ve cases
1	IDC	13	23–74	7	6	3–6
2	Medullary carcinoma	1	45	0	1	<3
	Total	14		7	7	

Table 4 shows that in 14 malignant cases, 13 were of IDC and one was of medullary carcinoma. Out of 13 cases 7 (53.84%) shows positive immunostaining with CK 5/6 and the staining pattern was fine granular cytoplasmic staining with staining score ranging from 3–6, except for 1 case that shows cytoplasmic as well as membranous staining and score was also from range 6–9, while 6 cases were negative for immunostaining with CK 5/6 and the staining was <3. Only one case of medullary carcinoma was also immunostaining negative with staining score <3.

Table 5: Correlation of malignant cases with tumor size, necrosis, axillary lymph node metastasis and grade of tumor.

Clinico-pathological variables	CK 5/6 positive cases (n=07)	CK 5/6 negative cases (n=07)	Total Cases (n=14)
Mean age (years)			
Mean age	48.14	50.66	49.0
Histopathological diagnosis			
IDC NOS	7(50%)	6 (42.8%)	13 (92.8%)
Medullary carcinoma	0	1 (7.14%)	1 (7.2%)
Tumor size			
Average size(cm)	5.7	4.3	5
Tumor necrosis			
Present	4 (57.14%)	2 (33.33%)	6 (46.15%)
Absent	3 (42.85%)	4 (66.66%)	7 (53.84%)
Lymph node metastasis			
Present	4 (57.14%)	1 (16.66%)	5 (38.46%)
Absent	3 (42.85%)	5 (83.33%)	8 (61.53%)
Grade of tumor			
Grade I	0	3 (42.85%)	3 (23.0%)
Grade II	3 (42.85%)	3 (42.85%)	6 (46.15%)
Grade III	4 (57.14%)	0	4 (30.76%)

Table 5 shows that Out of 14 cases 07 cases show positive immunostaining for CK 5/6. Average size of tumor with positive cases was 5.7 cm whereas for negative cases it was 4.3 cm. out of 07 immuno positive cases 04 (57.14%) cases show tumor necrosis whereas only 02 (33.33%) cases of immune-negative cases show tumor necrosis. Axillary lymph node metastasis found in 04 (57.14) cases of CK 5/6 positive cases, whereas only 01 (16.66%) case with negative immunostaining show lymph node metastasis. Most of cases that shows positive immunostaining with CK 5/6 was of grade III (57.14%) followed by grade II (42.85%), not a single case of grade I was found to be immune-positive. In case of immune-negative cases grade I tumors were also found.

Table 6: Comparison of CK 5/6 positive cases in benign breast disease.

	Akhtar et al	Bhalla et al	Jain et al	Present study
Fibroadenoma	20/20 (100%)	9/9 (100%)	27/27 (100%)	22/22 (100%)
Fibrocystic	6/6 (100%)	12/12 (100%)	12/12 (100%)	4/4 (100%)
Lactating adenoma	0	0/1 (0%)	0/2 (0%)	0/0
UDH	4/4 (100%)	0/0	6/6 (100%)	0/0
Duct ectasia	8/8 (100%)	1/1 (100%)	3/3 (100%)	0/0
Total	38 (100%)	22/23 (95.6%)	48/50 (96%)	26/26 (100%)

Table 6 shows that the present study 100% of the benign breast disease show positive immunostaining with CK 5/6 just like other studies of Akhtar et al, Bhalla et al and Jain et al.

Table 7: Comparison of CK 5/6 positive cases in malignant breast disease.

	Akhtar et al	Bhalla et al	Jain et al	Present study
DCIS	0/18	0/3	0/11	0/0
IDC	6/22	6/22	18/38	7/13
Medullary carcinoma	0/0	0/0	1/1	0/1
Total	40	25	50	14

Table 7 shows that in present study majority of cases belongs to IDC. Out of 13 cases 07 cases show positive immunostaining with CK 5/6. Similar results were also seen in different studies e.g. Akhtar et al, Bhalla et al and Jain et al. Only one case of medullary carcinoma was found immune-negative in present study, although Jain et al found immune-positivity but with low staining index.

Among 40 cases of breast disease studied presently, 26 (65%) cases were of benign lesions of breast and 14 (35%) were malignant breast carcinoma. All the benign cases of breast showed positive CK 5/6 expression with variable staining score.

Immunostaining in fibroadenoma cases

Fibroadenoma showed proliferating breast ducts

with predominantly fibrous stroma around dilated and compressed ducts with cytoplasmic and membranous CK 5/6 positivity in the outer myoepithelial cell layer only.(Fig.1.1)

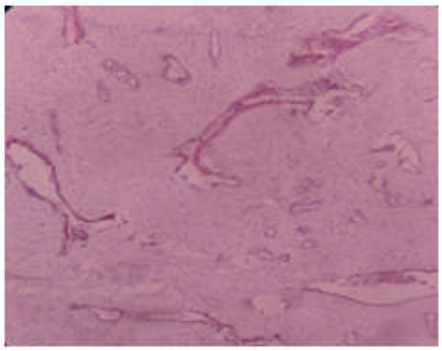

Fig 1.1 (IHC): Immunohistochemical staining with cytokeratin 5/6 in a case of fibroadenoma (H&CE, 100x).

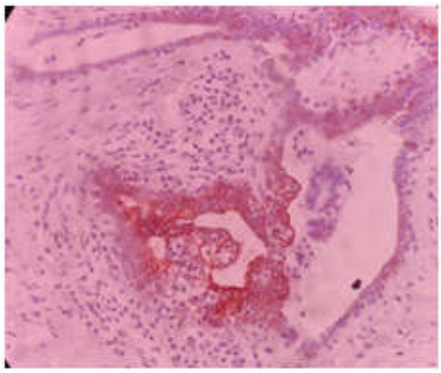

Fig. 1.1(IHC): Immunohistochemical staining with cytokeratin 5/6 in a case of fibroadenoma (H&E, 400x)

Immunostaining in fibrocystic disease cases

Fibrocystic disease showed cystically dilated ducts lined by cytoplasmic and membranous CK 5/6 positive flattened epithelium with mild pericystic fibrosis and inflammation.(Fig.1.2)

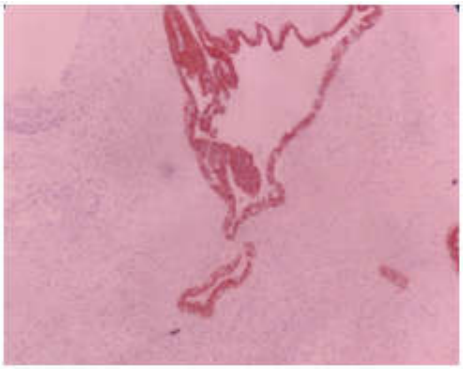

Fig. 1.2 (IHC): Immunohistochemical staining of cytokeratin 5/6 in case of fibrocystic disease of breast (H&E, 100x).

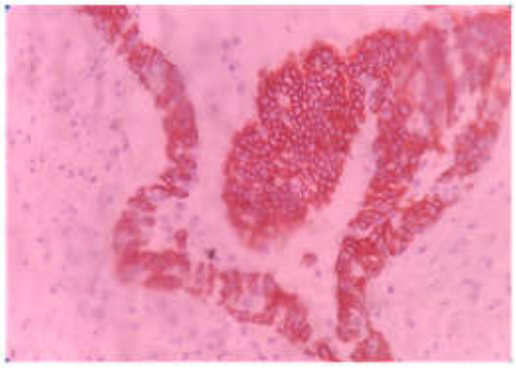

Fig. 1.2 (IHC): Immunohistochemical staining of cytokeratin 5/6 in case of fibrocystic disease of breast (H&E, 400x).

Immunostaining in invasive ductal carcinoma cases

Out of 14 cases of malignant breast disease including majority of cases of IDC (13 cases), the 6 cases showed negative CK 5/6 immunoreaction in luminal cells and faint fine granular cytoplasmic staining in basal cells with staining score <3 while 7 cases showed positive cytokeratin expression with staining score 3–6.(Fig.1.3)

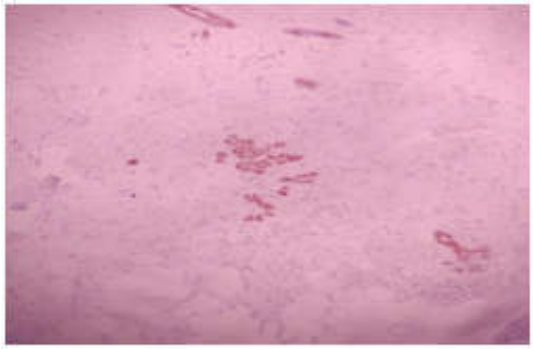

Fig. 1.3 (IHC): Immunohistochemical staining with cytokeratin invasive ductal carcinoma (H&E,100x).

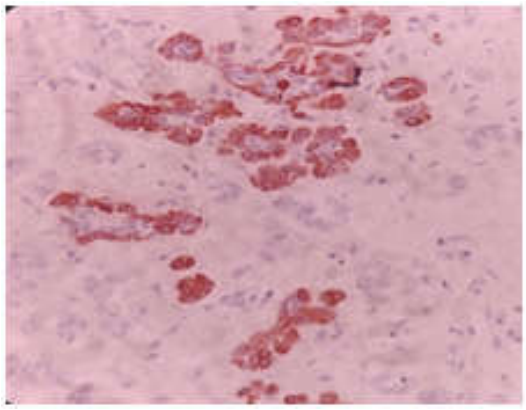

Fig 1.3 (IHC): Immunohistochemical staining 5/6 in case of with cytokeratin 5/6 in case of IDC (H&E, 400x).

Immunostaining in Medullary carcinoma cases

One case of medullary carcinoma also showed negative expression of cytokeratin with fine granular cytoplasmic staining with staining index <3.

The mean age of presentation in 6 cases of negative cytokeratin expression of invasive ductal carcinoma and 7 cases of positive cytokeratin expression of invasive carcinoma were 50.6 years and 48.14 years respectively. Axillary lymph node metastasis was positive in 4 (57.14%) cases and negative in 3 cases out of the 7 cases with positive immunostaining. Only 1 (16.66%) case of immune-negative cases of IDC showed lymph node metastasis. Average tumor size for CK 5/6 positive expression of IDC was 5.7 cm and 4.3 cm for negative CK 5/6 expression cases of IDC. Tumor necrosis was found in 4 (57.14%) cases out of 7 cases of CK 5/6 positive expression, only 2 (33.33%) cases out of 6 cases with negative CK 5/6 expression showed tumor necrosis.

CK 5/6 positive expression was found in grade III (57.14%) tumors predominantly followed by grade II (42.85%) tumors. Not a single case of IDC with grade I was found CK 5/6 positive.

Discussion

The normal breast tissue consists of the following five distinct cell populations: Committed stem (progenitor) cells (CK5+), glandular precursor cells (CK 5+,CK 8+,18+,19+), glandular end cells (CK 8+,18+,19+), myoepithelial precursor cells (CK 5/6+,SMA+) and myoepithelial end cells (SMA+).[6]

CK 5 positive cells represent progenitor cells for both glandular and myoepithelial lineages of mammary epithelium. There is a gradual decrease of CK 5 expression, associated with an increase in expression of CK 8,18,19 in the glandular cells, and smooth muscle actin (SMA) in the myoepithelial cells along the pathways of differentiation. The terminally differentiated cells do not express CK 5. In the lactating breast there is segregation of epithelial structures into CK 8,18 expressing secretory zone and the proliferative zone which harbors cells of both glandular (CK 8,18+) and basal/myoepithelial (CK 5/6+) type. Lactating adenoma is a benign tumor comprised of terminally differentiated secretory cells and is thus CK 5/6 negative.[10]

The proliferated luminal cells in benign lesions show a large number of CK 5/6 positive cells because of proliferation of both glandular and basal cells. Most malignancies are derived from differentiated glandular cells and do not reveal immune-histochemical staining with CK 5/6 thus explaining its negativity in most lesions of atypical hyperplasia and ductal carcinoma in situ.[6,11]

Breast cancer is traditionally classified based on the histological type of the tumor. The decision about the treatment and prognostication is largely based on certain clinicopathological parameters like tumor size, lymph node stage, histological type, tumor grade, vascular invasion and patient's age.[12]

Currently four main molecular classes of breast carcinoma are recognized namely, luminal, Her-2 enriched, basal like and normal like. Amongst these, basal like is the most aggressive with poor prognosis and reduced survival. It is resistant to existing molecularly-targeted treatment modalities.[12,13,14]

Cytokeratin 5/6 being basal markers, essentially identifies majority of the basal like tumors. In addition, the gene expression profile (GEP) of CK 5/6 positive breast carcinoma is said to be like that of BRCA1 mutation positive tumors. This warrants the screening of BRCA1 gene in patients having CK 5/6 positive tumors.[2,15,16]

Breast cancer has been classified into distinct molecular subtypes based on gene expression profiling studies. Each subtype is a distinct entity with clinical, biological and therapeutic implications. The basal like group of tumors possess an expression signature similar to basal/ myoepithelial cells of breast and is reported to have transcriptomic similarities with tumors arising in breast cancer 1 (BRCA1) germline mutation carriers.

Basal like breast cancers are poorly differentiated, high grade infiltrating carcinomas with large central acellular areas. They are an indicator of aggressive behavior and poor prognosis, independent of nodal status and tumor size. They show a specific pattern of distant metastasis with an increased propensity for visceral metastasis to brain and lung.[12]

In the Present study 40 cases were taken. Amongst 40 cases, 26 benign cases, maximum were of fibroadenoma (55%), showed 100% immunopositivity for CK 5/6 with high staining index (6–9) in myoepithelial cells and glandular epithelium with membranous and cytoplasmic staining of varying degree. Out of 14 cases maximum 13 (32.5%) were invasive ductal carcinoma and 01 (2.5%) case of medullary carcinoma, 07 cases

of IDC were CK 5/6 positive with fine granular cytoplasmic staining with staining index 3–6, except in one case that showed membranous staining also. These findings are similar to the other studies.

Yanping D et al (2006)(7) studied 38 cases of benign breast lesions, positive rate of the CK 5/6 expression was 100%. In all cases, positive expression of varying degrees was seen in myoepithelial cells and glandular epithelium. In 5 cases of atypical ductal hyperplasia, there were only few positive cells in the ducts. In 19 cases of DCIS, no tumor expressed CK 5/6. In 19 cases of invasive ductal carcinoma, no CK 5/6 expression was observed.

Bhalla et al (2010)[2] studied 23 cases of benign and 25 cases of malignant breast lesions for immunostaining of CK 5/6.

All benign lesions showed positive immunoreaction, with the staining index varying from 6–9, except lactating adenoma. The malignant lesions comprised 03 cases of ductal carcinoma in situ (DCIS) and 22 cases of infiltrating ductal carcinoma, not otherwise specified, IDC (NOS). None of the DCIS cases showed a positive immunoreaction. Among the IDC (NOS) lesions, 06 cases of grade III breast carcinoma exhibited a positive immunohistochemical reaction, the staining index of which varied from 2–6. The staining reaction in malignant lesions was only cytoplasmic and the intensity was significantly less than that of benign lesions.

Akhtar Kafil et al (2015)[8] studied 78 patients of breast lesions. Amongst the 78 cases of breast lesions, 38 (48.7%) cases were benign breast disease, 18 (23.1%) were ductal carcinoma in situ and 22 (28.2%) cases were of malignant breast carcinoma. Out of 22 cases of malignant breast disease, 16 (72.7%) cases show negative cytokeratin reaction with staining score of <2 and 6 (27.3%) cases of triple negative breast carcinoma (TNBC) showed positive cytokeratin expression with staining score of 5–8.

Jain et al (2018)[9] studied 100 cases of breast neoplasm out of which 50 were benign and 50 were malignant. Maximum number of benign cases were fibroadenoma (54%). All the benign lesions were positive for CK5/6 with the staining index 6–9 except for two cases of lactating adenoma.

There were 38 cases of IDC, 11 cases of DCIS and 1 case of medullary carcinoma. All the cases of DCIS were immune negative for CK5/6 staining. 18 cases of IDC and 1 case of medullary carcinoma showed positivity for CK5/6 but had staining index less than benign lesions in most of the cases. Maximum CK5/6 positive cases of infiltrating carcinoma had age below 50 years (89%). 75% of triple negative cases were CK5/6 positive and 63% cases of CK5/6 positive infiltrating carcinoma were triple negative.

Conclusion

Benign lesions shows strong immunohistochemical staining with CK5/6 in myoepithelial layer. With the help of IHC marker CK5/6 we can differentiate benign lesions from malignant. Malignantlesions showing staining with CK5/6 have weak positivity, but they are basal type according to molecular classification and have an aggressive behavior and poor response to therapy.

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