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Pathology

Role of Cytokeratin 5/6 Expression for Diagnosis and Prognosis of Basal Like Molecular Subtype of Malignant Breast Lesions

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Abstract **Original Research Article**

Background: Cytokeratin 5/6 expression by basal/myoepithelial cell in epithelial breast lesion can be used to identify myoepithelial cells. ***Methods:*** In the present study immunohistochemical staining for cytokeratin 5/6 was done on 51 cases of malignant epithelial breast lesions by using avidin biotin peroxidase technique on paraffin embedded sections. Distribution and intensity of immunostaining for cytokeratin 5/6 in various malignant breast lesions were recorded. ***Result***: Among 51 malignant breast lesions, 12 cases (23.52%) showed positive immunostaining with cytokeratin 5/6 with staining index ranging from 2-7, 1 case of Ductal carcinoma in situ which show negative immunostaining with cytokeratin 5/6 and 5 out of 8 cases (62.5%) of triple negative infiltrating carcinoma showed positive immunostaining with cytokeratin5/6. ***Conclusion:*** Cytokeratin 5/6 antibody is used to detect basal like carcinomas in malignant breast lesions to correlate with the prognosis and treatment of breast carcinoma.

Keywords: Basal like breast cancer, Triple negative breast cancer, Cytokeratin 5/6.

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INTRODUCTION

On basis of molecular weight, 20 members of human CKs family defined[1,2] of which 16 CKs (1-8,10,11 and 14-19) have been immunohistochemically or biochemically identified in normal or malignant breast epithelial cells [1-3]. The normal breast tissue consists of the following five distinct cell populations. Committed stem (progenitor) cells (CK5+), glandular precursor cells (CK5+/CK8/18/19+), glandular end cells (CK8/18/19+), myoepithelial precursor cells (CK5/6+/SMA+) and myoepithelial end cells (SMA+)[4]. The basal/myoepithelial cells express cytokeratin 5/6 and thus can be used to identify myoepithelial cells in various tissue sections [5].

On basis of analysis of gene expression profiling data, breast cancers have different "molecular subclasses" that have prognostic significance. The "basal-like" breast cancers, one of these molecular subclasses that have been associated with worse prognosis compared to luminal subtypes. The "basal-like" breast cancers, express IHC marker CK5/6[6] and highly associated with BRCA1 mutation [7].

Triple negative breast cancer are those tumors which are Estrogen receptor (ER), Progestrogen receptor (PR) and HER-2-new negative, account for 15-20% of newly diagnosed breast cancer. Triple negative breast cancer also comprise basal like molecular subtype. However triple negative breast cancer and "basal-like" breast cancers are not synonymous. For example in a study by IHC analysis noted that 71% of triple negative breast cancers were found to be of "basal-like" breast cancers and conversely 77% of "basal-like" breast cancer were triple negative[8].

MATERIAL AND METHODS

For the immunohistochemistory study purpose paraffin embedded sections were used. All cases of malignant breast lesion diagnosed during a period of 1 year (Jan 2018 to December 2018) were taken. Cytokeratin 5/6 immunostaining done on all cases. Then positive cases grouped in one, negative cases grouped in other group. Then each group correlated with histological type of malignancies, mean age, immunoscore, tumor size, tumor stage, nodal

metastasis, lymphovascular invasion, necrosis etc (Table 4).

Materials for IHC

Primary and secondary antibody kits, Antigen retrieval solution, Wash buffers, distal water, hematoxyline etc.

Procedure for IHC

For Dewaxing slides were kept in oven at 60-70◦C temperature for 30-40 minutes. After that, 3 changes of Xylene for 07 minutes each. Followed by 2 changes of Absolute alcohol for 5 minutes each. For antigen retrieval Heat induced epitope retrieval (HIER) is used.

Cytokeratin 5/6 immunostaining score is calculated by adding score of the two categories given below

- Intensity score - Intensity of staining
- Proportion score – Proportion of cells having immunopositivity

Table-1

Intensity score	Proportion score
0=No staining 1=Weak staining 2=Moderate staining 3=Strong staining	1=<10% positive staining 2=10-25% positive staining 3=26-33% positive staining 4=34-66% positive staining 5=67-100% positive staining

Maximum score – Not >8
Score <2 – Cytokeratin 5/6 Negative
Score >2 – Cytokeratin 5/6 Positive

STATISTICAL ANALYSIS

Data so collected was tabulated in an excel sheet. Data were analysed using IBM SPSS. Statistics Windows, Version 20.0. The statistical significant difference among groups was determined by the chi square test and fisher exact test. The level of significance was set at p <0.05.

RESULT

A total of 51 cases of malignant breast lesions were studied. Infiltrating ductal carcinoma not otherwise specified (IDC-NOS) was the most common (90.2%) histological type of malignant breast lesions. 15.21% of infiltrating ductal carcinoma not otherwise specified (IDC-NOS) showed ductal carcinoma in-situ (DCIS) component. Table 2 showing cytokeratin 5/6 expression in various malignant breast lesions. Common age group for malignant breast lesions was above 40 years of age. (68.63%). Mean age at diagnosis in cytokeratin 5/6 immunopositive cases in malignant breast lesions was 52.3 years. Table 3 showing age wise distribution in various malignant breast lesion. The only single case of Ductal carcinoma in-situ (DCIS) that was found cytokeratin 5/6 negative. Out of 46 Infiltrating ductal carcinoma-not otherwise specified (IDC-NOS) cases, 10 cases (21.73%) showed positive immunostaining for cytokeratin 5/6 (p=0.139). Malignant breast lesions show less immunostaining score that were ranging from 2-7. Majority of cytokeratin 5/6 immunopositive malignant breast lesions presented with T2 stage (p=0.481). Mean tumor size in cytokeratin 5/6 immunopositive malignant breast lesions was 4cm. 75% of cytokeratin 5/6 immunopositive malignant breast lesions showed lymph node metastasis (p=0.090). 91.67% of cytokeratin 5/6 immunopositive malignant breast lesions had histological grade II and III (p=0.0005). 75% of cytokeratin 5/6 immunopositive malignant breast lesions showed lymphovascular invasion (p=0.040). 66.67% of cytokeratin 5/6 immunopositive malignant breast lesions showed perineural invasion (p=0.003). 66.67% of cytokeratin 5/6 immunopositive malignant breast lesions had necrosis (p=0.04). 62.5% of triple negative malignant breast lesions showed positive immunostaining for cytokeratin 5/6.

Table-2: Cytokeratin 5/6 expression in various malignant breast lesions

Diagnosis	Total no. patients	Positive	Negative
DCIS	1	0	1
IDC-NOS	46	10	36
Lobular carcinoma	1	0	1
Medullary carcinoma	1	0	1
Metaplastic carcinoma	1	1	0
Intracystic Papillary carcinoma	1	1	0
Grand Total	**51**	12	39

Table-3: Age wise distribution in malignant breast lesions

Age (years)	No. of patients	Percentage
21-30	6	11.76
31-40	10	19.61
41-50	9	17.65
51-60	13	25.49
61-70	6	11.76
71-80	7	13.73

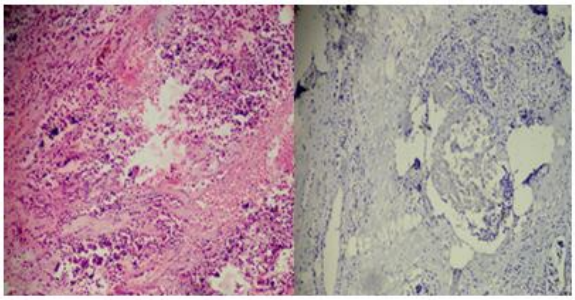

Fig-1: IDC- NOS (a=x10 H&E stain section, b= x10 CK5/6)

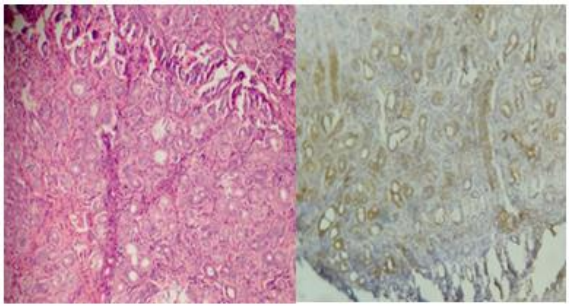

Fig-2: IDC-NOS (a=x10 H&E, b=x10 CK5/6)

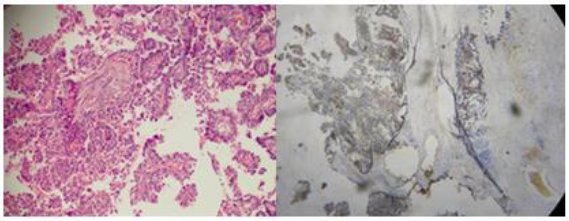

Fig-3: Intracystic Papillary carcinoma of breast (a=x10 H&E, b=x4 CK5/6)

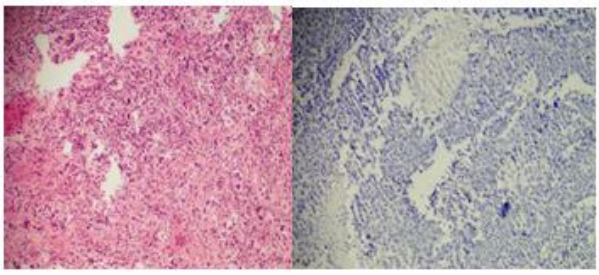

Fig-4: Lobular carcinoma of breast (a= H&E, x10. b=x10 CK5/6)

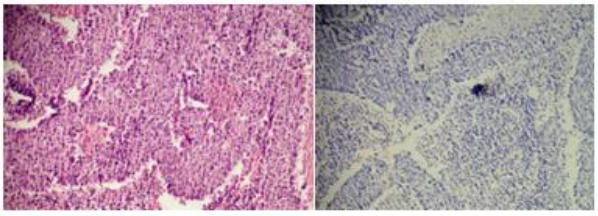

Fig-5: Medullary Carcinoma of breast (a=x10 H&E, b= x10 CK 5/6)

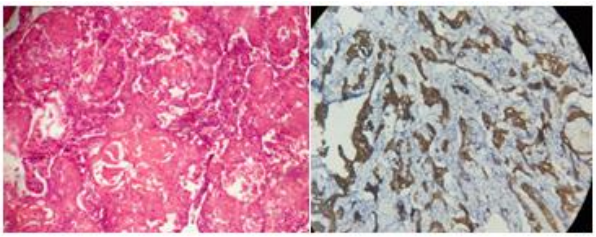

Fig-6: Metaplastic carcinoma of breast (a= x10 H&E, b= X10 CK5/6)

DISCUSSION

With the technique of tissue microarray (TMA) gene expression profiling (GEP) is being used nowadays for breast cancer prognosis, which aims at identifying the patient with reasonably good prognosis to allow the safe omission of adjuvant chemotherapy [9,10]. A study done by Sorlie *et al*. reported a distinctive molecular portrait of breast cancer 456 complementory DNA clones. They have derived tumors into five intrinsic subtypes with distinct clinical outcomes (Table 4).

Table-4

Intrinsic subtype	IHC status	Grade	Outcome	Prevalence[11]
Luminal A	ER+, PR+,HER2-,Ki67-	½	Good	23.7%
Luminal B	ER+,PR+,HER2-,Ki67+	2/3	Intermediate	38.8%
	ER+,PR+,HER2+,Ki67+		Poor	14%
HER-2 overexpression	ER-,PR-,HER2+	2/3	Poor	11.2%
Basal like	ER-,PR-,HER-2-,CK5/6+	3	Poor	12.3%
Normal like	ER+,PR+,HER-2-,Ki67-	1/2/3	Intermediate	7.8%

The rationale behind such molecular subtyping is that the difference underlying the gene expression pattern among cancer subtypes reflects positive fundamental difference at molecular level [12]. These

different subtypes expressing different gene profile are mapped to immunohistochemistory definer subtypes, which is the phenotype used for routine purposes is the gene expression profiling is not available everywhere upto no cost effective. We have focussed our study on triple negative tumors to find out the cytokeratin 5/6 (basal marker) positivity, incidence of basal like molecular subtype of breast cancer and its correlation with histological types, mean age, mean tumor size, tumor stage, nodal metastasis, lymphovascular invasion, necrosis etc. Basal like tumor accounts for 60-90% of triple negative cases [13,14]. They have an aggressive clinical course lack any standard targeted therapy. The patients are of young age. Various risk factors described early menarche, high waist to hip ratio, lack of breast feeding with high parity. Basal like molecular subtype is associated with lower disease specific survival and higher risk for local and regional relapse. The metastasis pattern of these basal like subtype is also differing from other tumors. As these tumor tends to involve the visceral organs specialy bone more than lymph nodes [15]. As these tumors are ER, PR and HER-2 negative, they donot respond to conventional targeted breast cancer therapies, so chemotherapy in form of anthracyclines and taxane are the only option left. In study by Chang HY *et al*. [16] shown that matrix remodelling and angiogenesis are associated with basal tumors, suggesting these avenues for targeting.

21.73% of Infiltrating ductal carcinoma showed positive immunostaining for cytokeratin 5/6 that implies "basal-like" phenotype. In our study most of CK5/6 positive malignant breast lesions occurs at early age onset (mean age 52.3years), less immunostaining score, mean tumor size is 4cm, high histological grade II and grade III. 75% cytokeratin 5/6 malignant breast lesions, showed lymph node metastasis, lymphovascular invasion and necrosis. Comparison with various other studies was done in table 5 given below.

Table-5

Parameter for comparison	**Present study 2018**	**Dent *et al.* [17]**	**Fadare O *et al.* [18]**	**Pinten *et al.* [19]**	**Bhalla *et al.* [20]**	**Pillai *et al.* [21]**	**Akhtar *et al.* [22]**	**Hashmi *et al.* [23]**
CK5/6 positivity (%)		-	17	-	24	-	27.3	8
Mean age (Years)	52.3	-	-	-	50	45.3	53	48.9
Immunoscoring range	2-7	-	-	-	2-6	-	1-6	
Mean tumor size (cm)	4	-	-	-	3	-	3	
Tumor stage	T2				T2		T2	T2
Nodal metastasis (%)	75	55			66.67		66.67	33.3
Histological grade III (%)	50	-	50	-	100	-	100	91.7
Lymphovascular invasion (%)	75	45	-	-	-	-	83.3	8.3
Necrosis (%)	75	-	-	-	100	-	100	83.3
CK5/6 positive in TNBC (%)	62.5	-	-	60	-	72.2	100	8

One case of Intracystic Papillary carcinoma, showed positive for cytokeratin 5/6 immunostaining. One case of medullary carcinoma, showed negative immunostaining for cytokeratin 5/6. However Tot *et al*. [24] reported that 25% of the typical, 43% of the atypical and 20% of the metastatic medullary carcinomas, showed cytokeratin 5/6 immunopositivity. One case of Lobular carcinoma, showed negative immunostaining for cytokeratin 5/6. However Fadare O *et al*. [18] reported that17% of Lobular carcinoma express cytokeratin 5/6. One case of Metaplastic carcinoma, showed strong positive immunoscoring that was 7 for cytokeratin 5/6. Rungta S *et al*. [25] also reported that cytokeratin 5/6 expression was the most sensitive for metaplastic carcinoma. One case of Intracystic papillary carcinoma (IPC) and one case of metaplastic carcinoma were positive for cytokeratin 5/6 immunostaining.

CONCLUSION

By positive cytokeratin 5/6 expression in various malignant breast lesions, "basal-like" breast cancers are detected that are likely to be high grade invasive tumor, early age of onset and have overall poor prognosis. The "basal-like" breast cancers are highly associated with BRCA1 mutation, thus patient of "basal-like" breast cancers along with their first degree relative must be advised for BRCA1 mutation testing. This study further concluded that there are certain tumor which is triple negative, cytokeratin 5/6 negative and Ki 67 negative, these canot be subtyped. In present subtype system such cases needs further studies to ascertain its prognosis and treatment stratagies.

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