Original Article

DOI: 10.7860/JCDR/2022/55331.16723

Pathology Section

Estrogen Receptor, Progesterone Receptor Profile in Association with CK 5/6 Immunohistochemical Status in Proliferative, Preinvasive and Malignant Epithelial Neoplasms of Breast

SNEHA[1], TUSHAR KANTI DAS[2]

ABSTRACT

Introduction: Breast carcinomas have shown increasing incidence across the world over the recent few years. The different epithelial cells play a role in the pathogenesis of different breast lesions consistent with the varying cytokeratin (CK) expression profiles. The luminal cells express CK 8 and 18 while myoepithelial cells show CK 5/6 and CK 17 expression. Triple Negative Breast Cancers (TNBC) (hormonal receptors and Human Epidermal growth factor Receptor 2 (HER2)/neu negative) express basal cytokeratins and histopathologically show metaplastic to medullary features while luminal breast cancers with glandular differentiation show hormonal receptor or HER2 expression. Also basal cells are characteristic of benign lesions like epithelial hyperplasia, fibroadenoma etc. while being absent in atypical hyperplasia and preinvasive lesions.

Aim: To study cytokeratin 5/6 and Estrogen Receptor/Progesterone Receptor (ER/PR) expression pattern in proliferative, preinvasive and malignant lesions of breast.

Materials and Methods: An observational cross-sectional study was undertaken in the Department of Pathology in a tertiary care hospital in East India, from January 2019 to June 2020. A total of 41 samples diagnosed as proliferative (Usual Ductal Hyperplasia (UDH)/Atypical Ductal Hyperplasia (ADH), preinvasive Ductal carcinoma in Situ (DCIS) and invasive breast carcinomas were selected by systematic random sampling. Immunohistochemical examination was done using monoclonal antibodies against Cytokeratin 5/6 and ER/PR/HER2 after obtaining thin sections from formalin fixed paraffin embedded blocks and retrieval of antigen. The data was interpreted by light microscopy using a semi-quantitative method with respect to prefixed parameters and statistical analysis was done by Chi-square test and Fischers-exact test using Statistical Package for the Social Science (SPSS) version 25.0.

Results: Of the 41 cases, three were of proliferative lesions (UDH+ADH), 1 (33%) (UDH) showed positive CK 5/6 expression and 2 (66.7%) (ADH) showed negative CK 5/6 expression. Of two preinvasive lesions (DCIS), 100% of them showed negative CK 5/6 expression. On categorisation of carcinoma cases into molecular subgroups as indicated by surrogate immunohistochemical expression, it was found that majority of the cases (20) exhibited Luminal-A Like molecular profile constituting 55.6% of total. This was followed by an equal incidence of HER2/neu enriched (non luminal) and triple negative phenotypes. Both Luminal B-like (HER2-positive) and Luminal B-like (HER2-negative) were three in number contributing to 8.3% of total each. Out of 36 malignant cases, 5 (13.9%) showed positive CK 5/6 expression while 31 (86.1%) showed negative CK 5/6 expression. All these five cases showing positive CK 5/6 expression belonged to triple negative molecular subtype and this association between the molecular subtypes and CK 5/6 expression pattern was statistically significant p-value=0.0034. Of total five TNBC cases, 2 (40%) were reported to have weak positive CK 5/6 immunostaining, while 3 (60%) of the cases had moderate intensity. Still none of these cases exhibited strong immunostaining. The single UDH case reported in present study, exhibited strong positive immunostaining with CK 5/6.

Conclusion: The proliferative lesions consisting of both luminal and myoepithelial cells like UDH showed strong membranous and cytoplasmic expression while ADH, DCIS, and invasive breast carcinoma comprising primarily of luminal epithelial cells were negative for basal cytokeratin 5/6 expression. These group of breast carcinomas belonged to other immunophenotype categories apart from TNBC. However, a special immunophenotype TNBC group, negative for ER/PR and HER2/neu was strikingly positive for CK 5/6 and a statistically significant association was found.

Keywords: Cytokeratins, Invasive ductal carcinoma, Not otherwise specified, Triple negative breast cancers

INTRODUCTION

As per the statistics revealed by (GLOBOCAN), 2018 breast cancer has leading incidence in females followed by colorectal and lung carcinomas [1]. The breast ducts contain luminal cells with a role in pathogenesis of atypical hyperplastic lesions, preinvasive and invasive lesions while basal cell differentiation is seen in benign lesions. The stem cells, precursor of both express CK 5 [2,3]. Myoepithelial cells show CK 5/6 and CK 17, while luminal cells have CK 8 and 18 expressions [4]. Consequently benign lesions express CK 5/6 while in situ and invasive carcinomas are devoid of basal cytokeratins [5,6] with simultaneous expression of luminal CK (CK 8 and 18).

The lack ER, PR and HER2/neu expressions and are categorised into basal and non basal subtypes. Out of which only, basal types (basal like breast carcinomas, BLBC) are C 5/6 and/or Epidermal Growth Factor Receptor (EGFR) positive [7]. The basal cytokeratin profile has been classified as four clusters with respect to CK 5 and CK 17 expression. Basal type cluster 1 (CK 5 and 17 negative), cluster 3 (CK 5 negative and CK 17 positive) have good while cluster 2 (CK 5 positive and CK 17 negative) have the worst prognosis [8]. Cluster 4 (CK 5 and 17 positive) behaves as an intermediate category.

The CK, an intermediate filament protein, a marker of epithelial differentiation is utilised for the fingerprinting of carcinomas in general [9]. Strikingly most of benign lesions are known to show positive immunoexpression for luminal CK except lactating adenoma [10]. Further the staining intensity in malignancy is weak and only

cytoplasmic [11]. To ascertain the diagnosis of solid papillary carcinoma in situ and Intraductal Papilloma with Usual Ductal Hyperplasia (IPUDH) a panel of markers comprising different high molecular weight cytokeratins-CK 5/6, CK14, and CK34betaE12 is of immense importance [12,13]. DCIS show remarkable variation in CK expression profile with respect to ER-α, PR and EGFR status suggesting the molecular heterogeneous pathways for DCIS in lines with carcinomas [14].

The p63, alpha Smooth Muscle Actin (SMA) and CK 5/6 in combination aid in diagnosis of ductal proliferation with propensity towards malignant changes and as a panel significantly increase the diagnostic yield [15,16]. The immunohistochemical markers act as surrogate marker for molecular subtyping of breast carcinoma and in turn help in guiding the treatment [17]. In order to personalise therapy and determine the progression, the hunt for new biological markers is still on [18]. The misleading histomorphological terminologies need be supplemented with molecular signature profiling to gain a breakthrough in management [19,20].

The luminal as well as basal carcinomas have wide range of morphological variations, often acting as a clue for molecular categorisation [21]. Though the high grade family of TNBC is well recognised, low grade family includes low grade triple negative breast neoplastic family salivary gland-like tumours of breast with the former showing genomic signatures similar to that of classical triple negative group paradoxically despite being of low grade however the latter shows absence of all of such genetic markers and the two categories owe identification pertaining to the differing outcomes [22]. The present study was done with an aim to study cytokeratin 5/6 and ER/PR expression pattern in proliferative, pervasive and malignant lesions of breast also correlation of data by using appropriate statistical methods.

MATERIALS AND METHODS

An observational, cross-sectional study was undertaken in the Department of Pathology in a tertiary care Institution of Kolkata from January 2019 to June 2020. Approval from an Institutional Ethics Committee was obtained at the initiation of the study Institutional Ethics Committee RG Kar Medical College (Reg No- ECR/322/Inst/WB/2013) Memo No- RKC/470(15.01.2019). A total of 41 samples diagnosed as UDH and ADH, DCIS and invasive carcinomas were selected by systematic random sampling.

Inclusion criteria: Histopathological specimens received in the Department of Pathology within the study period (either received as tru-cut, lumpectomy or surgically excised specimen) morphologically diagnosed as proliferative, preinvasive and malignant epithelial lesions of breast were included.

Exclusion criteria: The specimens of non proliferative breast lesions (apocrine change, adenosis, fibroadenoma etc) and stromal tumours (phyllodes and mesenchymal tumours) were excluded from the study.

Study Procedure

Ultrathin (3-4 microns) sections are obtained by microtomy from the formalin fixed paraffin embedded blocks. After floatation they were picked on poly-L-lysine coated slides, dried, deparaffinised and rehydrated in descending grades of alcohol.

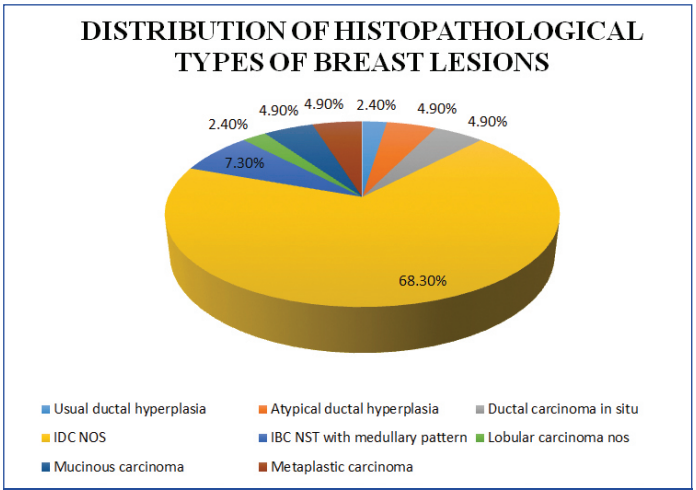
Heat Induced Epitope Retrieval (HIER) procedure was done by microwave method using Tris Hydroxymethyl Amino methane (TRIS) Buffer, EMPARTA, pH 9.0. TRIS Buffer (EMPARTA, pH 7.2) was used for washing. Endogenous peroxidase activity was blocked with poly excel Peroxidase Block, (Pathnsitu). Incubation with primary antibody: Rabbit Monoclonal antibody against CK 5/6, Pathnsitu cocktail EP24/ EP67, ER-Rabbit Monoclonal Antibody-Cell Marque, PR-Rabbit Monoclonal Antibody-Cell Marque was done at 37°C for 60 minutes. For visualisation of result, serial incubation for 30 minutes each was carried out with Poly Excel Target Binder, PATHNSITU; Poly Horse Radish Peroxidase (HRP) (Poly Excel HRP DAB Detection System, PATHNSITU) and chromogenic (Poly excel Stunn DAB Buffer and Poly excel Stunn Diaminobenzidine (DAB) Chromogenic, pathnsitu). The sections were then counter stained with Harris Haematoxylin and mounted. Sections of normal breast tissue were taken as control group. Proportional average expression of ER, PR, HER2/neu and CK 5/6 were allocated by semi-quantitative method using light microscopy, based on the overall impression, after scrutinising the whole slide especially focusing on the hot spot zones. Intensity of cytoplasmic and membranous immunostaining for CK 5/6 were graded as 1-3 (1:0-10%, 2:11-50%, 3:>50%) [12]. ER/PR expression were denoted as positive if >1% of tumour nuclei stained positively and negative when <1% or 0% stained positive for the same [23].

STATISTICAL ANALYSIS

The data was collected and analysed by Chi-square test and Fischers-exact test using SPSS, version 25.0.

RESULTS

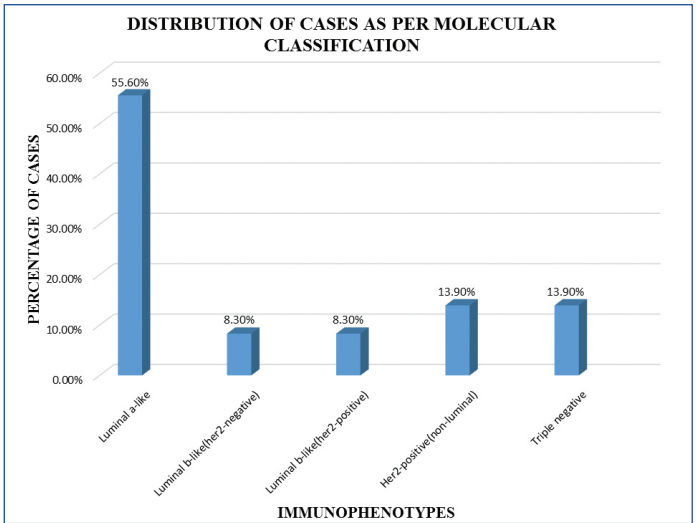
A total of 41 sample were taken which included spectrum of histopathological lesions ranging from proliferative, preinvasive and frankly malignant cases. Three cases belonged to proliferative lesions out of which one was UDH (2.4%) and two were ADH (4.9%). Two cases of DCIS constituting 4.9% of total. The majority of cases were Invasive Ductal Carcinoma (IDC), Not Otherwise Specified (NOS) making 68.3% of total (28). Of special types, single case of Lobular carcinoma NOS (2.4%) followed by two cases each of mucinous and metaplastic carcinoma were observed constituting 4.9% of total. Also three cases of IBC-NST with medullary pattern were noted (7.3%) [Table/Fig-1].



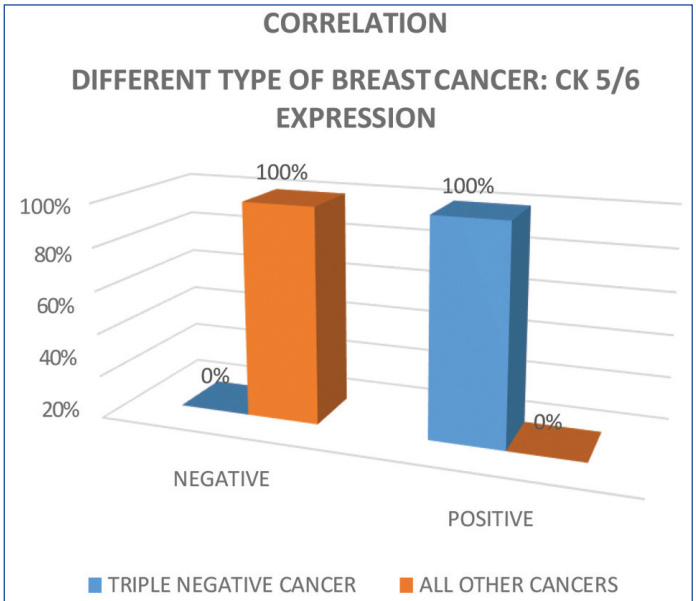
[Table/Fig-1]: Pie chart depicting distribution of spectrum of breast lesions.

On categorisation of carcinoma cases into molecular subgroups, it was found that majority of the cases (20) exhibited Luminal-A Like molecular profile constituting 55.6% of total. This was followed by an equal incidence of HER2/neu enriched (non-luminal) and triple negative molecular phenotypes, both being 05 in number (13.9%). Both Luminal B-like (HER2-positive) and Luminal B-like (HER2- negative) were three in number (8.3%) [Table/Fig-2].

Of total three proliferative lesions, 2 (ADH) of them (66.7%) were reported to be CK 5/6 negative while single case of UDH (33.3%) was reported to be CK 5/6 positive. Both DCIS cases (premalignant category) exhibited negative CK 5/6 expression. Of total 36 invasive cases, 31 (86.1%) of them were CK 5/6 negative, however five of the cases (13.9%) exhibited CK 5/6 positivity [Table/Fig-3].

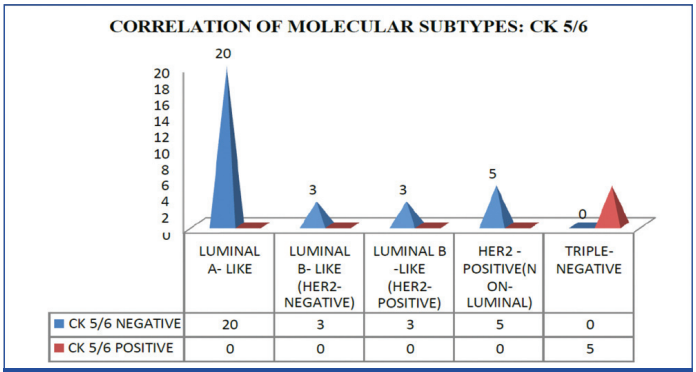


[Table/Fig-2]: Distribution of breast carcinoma cases as per the molecular classification.



[Table/Fig-3]: Showing association of CK 5/6 expression with different molecular phenotypes of breast carcinoma.

All of these five cases belonged to triple negative immunophenotypes and a statistically significant association existed between the molecular subtypes and CK 5/6 expression pattern as the p-value is 0.0034 (<0.05) [Table/Fig-4,5].

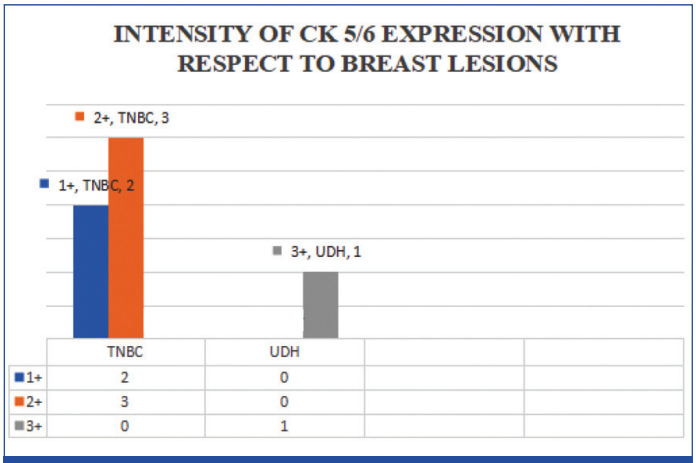


[Table/Fig-4]: A clustered pyramid chart showing association between molecular subtypes and CK 5/6.

Statistical method	Degrees of freedom	p-value	Impression
Fisher exact test	01	0.0034	Significant

[Table/Fig-5]: Statistical significance between molecular subtypes of IDC and CK 5/6 expression.

Of total, five TNBC, two of the cases (40%) were reported to have weak positive CK 5/6 immunostaining, while 03 (60%) of the cases had moderate degree of immunostaining with CK 5/6. Still none of these cases exhibited strong immunostaining. Further a single case of UDH case being reported in present study, exhibited strong positive immunostaining with CK 5/6 [Table/Fig-6]. Hispathological images of UDH, ADH, DCIS, is shown in [Table/Fig-7-10], immunohistochemical image of DCIS is shown in [Table/Fig-11], [Table/Fig-12,13] shows IDC, NOS. Invasive breast carcinoma, not otherwise specified (IBC NST) histopathology is shown in [Table/Fig-14,15].



[Table/Fig-6]: 3-D Clustered Column Chart highlighting the intensity of CK 5/6 expression in breast lesions.

DISCUSSION

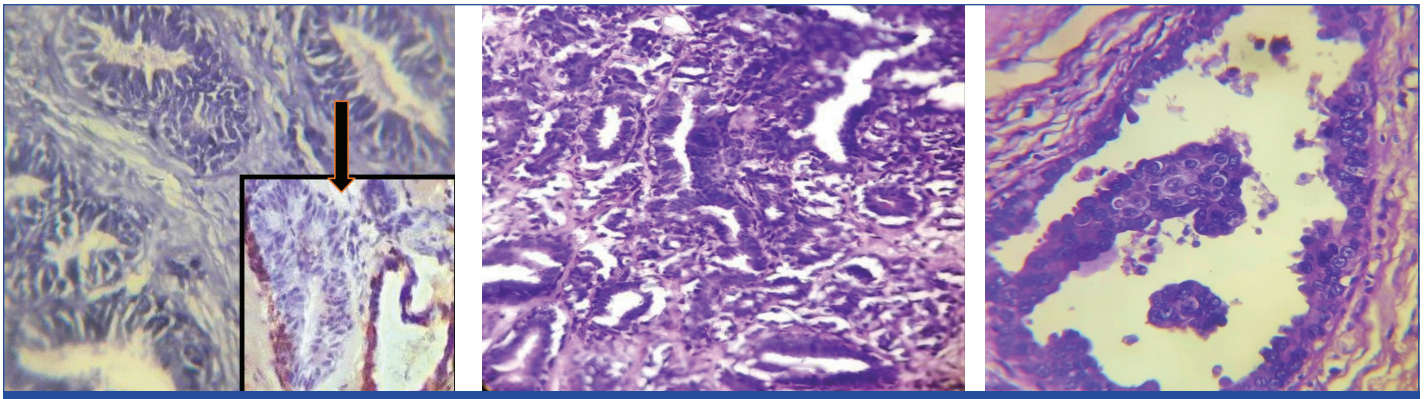
As per this study of 41 cases, one case of UDH was reported constituting 2.4% of total two cases each of ADH and DCIS were reported both constituting 4.9% each of total. 28 cases of, IDC, NOS were reported constituting 68.3% of total. One case of lobular carcinoma NOS was reported constituting 2.4 percent of total. Three cases of IBC-NST with medullary pattern were reported constituting 7.3% of total. Two cases each of mucinous and metaplastic carcinomas were reported both constituting 4.9% of total each [Table/Fig-1].

In this study, out of three cases of proliferative lesions (UDH+ADH), one of the case (UDH) constituting 33.3% of total showed positive CK 5/6 expression and two of the cases (ADH) mounting to 66.7% of total showed negative CK 5/6 expression of two preinvasive lesions, 100% of them showed negative CK 5/6 expression. However, statistically significant association could not be derived. Also Abdul EL et al., [5] and Raju U et al., [6] reported that atypical hyperplasia's and preinvasive lesions have negative CK 5/6 expression.

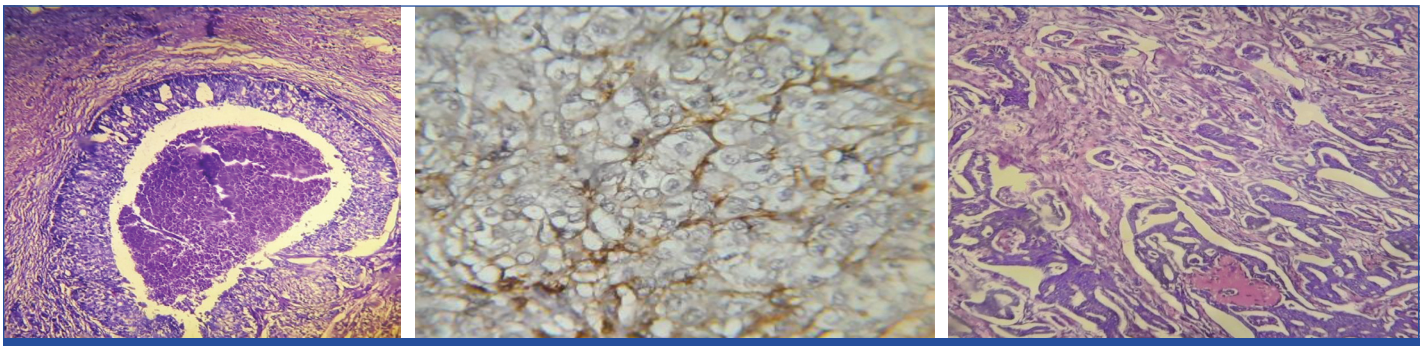
In a study by Lacroix-Triki M et al., strongly positive CK 5/6 expression was noted in all the lesions of UDH, 4 out of 5 cases of ADH showed <5% immunostaining while single case demonstrated 30% positivity. None of the LCIS/DCIS cases were reported to have positive expression [24]. Akhtar K et al., study showed 100% of UDH cases to demonstrate positive CK 5/6 expression while none of the DCIS cases had shown positive immunoreaction [25], in accordance with present results and hence it can conclude CK 5/6 has an important role in distinguishing the two lesions.

Out of 36 malignant cases, five of them showed positive CK 5/6 expression constituting 13.9% of the total while most of them showed (31) negative CK 5/6 expression constituting 86.1% of total. All these cases with positive CK 5/6 expression were triple negative on immunohistochemical analysis [Table/Fig-3,4]. The present study result showed concordance with previous studies like Mohammadisadeh F et al., showing same results with respect to CK 5/6 [4].

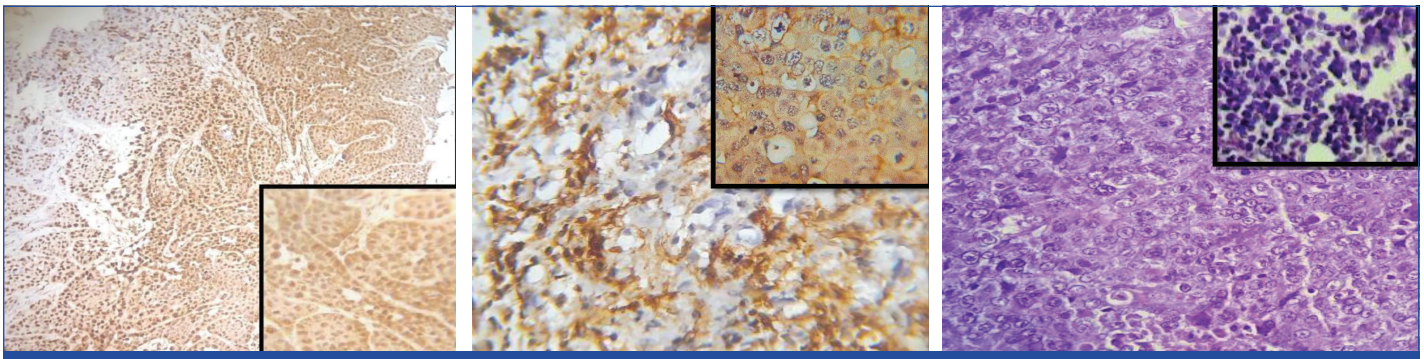
Also association between different molecular subtypes and CK 5/6 expression showed statistical significance as p-value came to be 0.0034 which was less than 0.05 [Table/Fig-5].



[Table/Fig-7]: Photomicrograph shows usual ductal hyperplasia, H&E (400X); Inset: CK 5/6 expression by myoepithelial cells (400 X) on immunohistochemistry.
[Table/Fig-8]: Photomicrograph shows Atypical ductal hyperplasia; H&E (400X). **[Table/Fig-9]:** Photomicrograph shows micropapillary DCIS; H&E (400X). (Images from left to right)



[Table/Fig-10]: Photomicrograph shows comedo pattern DCIS; H&E (400X). **[Table/Fig-11]:** Photomicrograph shows DCIS, CK 5/6 negative; (400X) on immunohistochemistry.
[Table/Fig-12]: Photomicrograph shows IDC, NOS; Bloom Richardson grading grade-2: H&E (100X). (Images from left right)



[Table/Fig-13]: Photomicrograph shows IDC NOS, ER positive (100X); Inset PR positive (400X) on immunohistochemistry. **[Table/Fig-14]:** Photomicrograph shows IBC NST with medullary pattern, CK 5/6 positivity (100X); Inset: mitotic figures (400X) on immunohistochemistry. **[Table/Fig-15]:** Photomicrograph shows IBC NST with medullary pattern, BR grade 03: Inset; tumour infiltrating lymphocytes (TIL), H&E (400X). (Images from left right)

The findings of this study strongly corroborated with Bhalla A et al., study where 22 cases of IDC and three cases of DCIS were evaluated for the expression of CK 5/6 and it was found that none of the DCIS cases stained immunopositive for the same [11]. Also most of the carcinoma cases histologically being IDC, NOS (19) similarly did not show any immunoreaction while rest of them was immunopositive for CK 5/6. Thus the present study shows concordance with the above study.

Two cases (40%) out of total five triple negative cases were seen to have weak cytoplasmic (01-10%) CK 5/6 expression, however three cases (60%) of this group showed moderate cytoplasmic staining (11-50%) while none of them showed strong staining (>50%). Also one of the reported case of UDH showed strong membranous and cytoplasmic (>50%) CK 5/6 staining. However no statistical significance was reported as evidenced by p-value of 01 which is more than 0.05 [Table/Fig-6]. Invasive breast carcinoma, not otherwise specified (IBC NST) histopathology is shown in [Table/Fig-14,15].

Limitation(s)

The efficacy of immunomarkers to detect and prognostic the cases employed on few number of tru-cut biopsies for hyperplastic lesions cannot be reasonably extrapolated on to a larger representative sample. The comparison of staining indices of CK 5/6 could not be assessed properly since benign lesions were excluded as a part of exclusion criteria.

CONCLUSION(S)

The study conducted in this setting, has reinforced the fundamental utility of cytokeratin's to distinguish the benign and malignant lesions in breast. Besides this, the characteristic expression of high molecular weight cytokeratin's, in triple negative subtypes has been an area of special interest, particularly with special reference to the poor prognosis which needs aggressive and effective treatment; hence better modalities are yet to be explored. Hence to sum up, present study stands as a bridge between what we already know with regard to the application of cytokeratin's in broad categorisation of different breast lesions and the trending knowledge of advanced immunohistochemcial markers. Global opinion is tilted towards the background role of cytokeratin's acting as a baseline reference for evaluation of proliferative and preinvasive lesions of breast.

REFERENCES

[1] Jana D, Mandal S, Mukhopadhyay M, Mitra D, Mukhopadhyay SK, Sarkar DK. Prognostic significance of HER-2/neu and survival of breast cancer patients attending a specialized breast clinic in Kolkata, Eastern India. Asian Pacific Journal of Cancer Prevention. 2012;13(8):3851-55.

[2] Chu PG, Weiss LM. Keratin expression in human tissues and neoplasms. Histopathology. 2002;40:403-39.
[3] Foulkes WD, Stefansson IM, Chappuis PO, Bégin LR, Goffin JR, Wong N, et al. Germline BRCA 1 mutations and a basal epithelial phenotype in breast cancer. J Natl Cancer Inst (Bethesda). 2003;95:1482-85.
[4] Mohammadizadeh F, Naimi A, Rajabi P, Ghasemibasir H, Eftekhari A. Expression of basal and luminal cytokeratins in breast cancer and their correlation with clinicopathological prognostic variables. Indian J Med Sci. 2009;63(4):152-62.
[5] Abd El, Rehim DM, Pinder SE, Paish CE, Bell J. Expression of luminal and basal cytokeratins in human breast carcinoma. J Pathol. 2004;203:661-71.
[6] Raju U, Crissman JD, Zarbo RJ, Gottlieb C. Epitheliosis of the breast: An immunohistochemical characterisation and comparison to malignant intraductal proliferations of the breast. Am J Surg Pathol. 1990;14:939-47.
[7] Sood N, Nigam JS. Correlation of CK5 and EGFR with clinicopathological profile of triple-negative breast cancer. Patholog Res Int. 2014;2014:141864.
[8] Alshareeda AT, Soria D, Garibaldi JM, Rakha E, Nolan C, Ellis IO, et al. Characteristics of basal cytokeratin expression in breast cancer. Breast Cancer Research and Treatment. 2013;139(1):23-37.
[9] Cooper D, Schermer A, Sun TT. Classification of human epithelia and their neoplasms using monoclonal antibodies to keratins: Straegies, applications, and limitations. Lab Invest. 1985;52:243-56.
[10] Böcker W, Moll R, Poremba C, Holland R, Van Diest PJ, Dervan P, et al. Common adult stem cells in the human breast give rise to glandular and my epithelial cell lineages: A new cell biological concept. Lab Investing. 2002;82:737-45.
[11] Bhalla A, Mridu M, Kahlon SK, Parbodh K, Nikita K. Cytokeratin 5/6 expression in benign and malignant breast lesions. Ind J Pathol Microbiol. 2010;53:676-79.
[12] Das A, Rashmi MV. Immunohistochemical study of CK 5/6 in Benign and malignant breast lesions. J Med Sci. 2018;4(4):95-102.
[13] Maeda I, Tajima S, Kanemaki Y, Tsugawa K, Takagi M. Use of immunohistochemical analysis of CK 5/6, CK14, and CK34betaE12 in the differential diagnosis of solid papillary carcinoma in situ from intraductal papilloma with usual ductal hyperplasia of the breast. SAGE Open Medicine. 2018;6:2050312118811542.
[14] Steinman S, Wang J, Bourne P, Yang Q, Tang P. Expression of cytokeratin markers, ER-alpha, PR, HER-2/neu, and EGFR in pure ductal carcinoma in situ (DCIS) and DCIS with co-existing invasive ductal carcinoma of the breast. Ann Clin Lab Sci. 2007;37(2):127-34.
[15] Ding Y, Ruan Q. The value of p63 and CK 5/6 expression in the differential diagnosis of ductal lesions of breast. Journal of Huazhong University of Science and Technology. 2006;26(4):405-07.
[16] Moriya T, Kasajima A, Ishida K, Kariya Y, Akahira JI, Endoh M, et al. New trends of immunohistochemistry for making differential diagnosis of breast lesions. Medical Molecular Morphology. 2006;39(1):08-13.
[17] Zaha DC. Significance of immunohistochemistry in breast cancer. World J of Clin Oncol. 2014;5(3):382.
[18] Aguiar FN, Mendes HN, Cirqueira CS, Bacchi CE, Carvalho FM. Basal cytokeratin as a potential marker of low risk of invasion in ductal carcinoma in situ. Clinics. 2013;68(5):638-43.
[19] Costarelli L, Campagna D, Mauri M, Fortunato L. Intraductal proliferative lesions of the breast-terminology and biology matter: Premalignant lesions or preinvasive cancer? Int J Surg Oncol. 2012;2012.
[20] Zhou W, Jirström K, Johansson C, Amini RM, Blomqvist C, Agbaje O, et al. Long-term survival of women with basal-like ductal carcinoma in situ of the breast: A population-based cohort study. BMC Cancer. 2010;10(1):653.
[21] Fadare O, Tavassoli FA. The phenotypic spectrum of basal-like breast cancers: A critical appraisal. Adv Anat Pathol. 2007;14(5):358-73.
[22] Geyer FC, Pareja F, Weigelt B, Rakha E, Ellis IO, Schnitt SJ, et al. The spectrum of triple-negative breast disease: High-and low-grade lesions. Am J Pathol. 2017;187(10):2139-51.
[23] WHO Classification of Tumors Editorial Board. Breast Tumors. Lyon (France). International Agency for Research on Cancer; 2019. (WHO Classification of tumors series, 5th ed.; vol 2) p 88.
[24] Lacroix-Triki M, Mery E, Voigt JJ, Istier L, Rochaix P. Value of cytokeratin 5/6 immunostaining using D5/16 B4 antibody in the spectrum of proliferative intraepithelial lesions of the breast. A comparative study with 34βE12 antibody. Virchows Archiv. 2003;442(6):548-54.
[25] Akhtar K, Bharduaj S, Naim M, Mansoor T, Sherwani RK. Diagnostic value of ck5 and ck6 in benign and malignant lesions of breast. Ann Pathol Lab Med. 2015;2(4):102-04.

PARTICULARS OF CONTRIBUTORS:
1. Senior Resident, Department of Pathology, R G Kar Medical College and Hospital, Kolkata, West Bengal, India.
2. Professor and Head, Department of Pathology, R G Kar Medical College and Hospital, Kolkata, West Bengal, India.

NAME, ADDRESS, E-MAIL ID OF THE CORRESPONDING AUTHOR:
Sneha,
BD-O75, First Floor, Salt Lake, Sector-1, Kolkata, West Bengal, India.
E-mail: snehajha1492@gmail.com

PLAGIARISM CHECKING METHODS: [Jain H et al.]
- Plagiarism X-checker: Feb 06, 2022
- Manual Googling: Apr 15, 2022
- iThenticate Software: May 09, 2022 (15%)

ETYMOLOGY: Author Origin

AUTHOR DECLARATION:
- Financial or Other Competing Interests: None
- Was Ethics Committee Approval obtained for this study? Yes
- Was informed consent obtained from the subjects involved in the study? Yes
- For any images presented appropriate consent has been obtained from the subjects. Yes

Date of Submission: **Feb 03, 2022**
Date of Peer Review: **Mar 18, 2022**
Date of Acceptance: **May 10, 2022**
Date of Publishing: **Aug 01, 2022**