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Original Article

Pathology Section

Role of CD10 as a Prognostic Marker in Invasive Breast Carcinoma

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ABSTRACT

Introduction: Stromal markers have been proved as important markers in assessing the prognosis of invasive breast cancer. Cluster of Differentiation (CD)10 is a cell surface enzyme with metalloendopeptidase activity which is expressed in stroma of various epithelial malignancies.

Aim: To estimate the expression of stromal CD10 expression in breast carcinoma and it's association with other prognostic markers like Oestrogen Receptor (ER), Progesterone Receptor (PR), Human Epidermal growth factor Receptor 2 (HER2-neu), Ki-67and tumour grade.

Materials and Methods: This study was a hospital based cross-sectional study conducted on 50 cases of breast cancer received in histopathology laboratory of the Department of Pathology, Government Medical College, Kottayam during a period of two years from July 2015 to June 2017. Adequate representative sections were taken and Haematoxylin and Eosin (H&E) staining was done. Immunohistochemistry (IHC) of the tissue sections was done by ER, PR, HER2-neu, Ki-67 and CD10. Descriptive statistics was used for the presentation of data showing number and percentages. Mantel-Haenzel (MH) Chi-square test was used for finding the association between CD10 and other prognostic markers of breast carcinoma like ER, PR, HER2-neu and Ki-67.

Results: In this study, all patients were females with mean age of 57.26 years. CD10 was found associated with tumour grade with p-value=0.013. The association of CD10 with ER, PR, HER2-neu was statistically insignificant. Though the association of CD10 and Ki-67 was observed (MH Chi-square value was 0.015), but it was not statistically significant (p-value=0.903).

Conclusion: A statistically significant association of CD10 with increasing tumour grade was observed and the association of CD10 with ER, PR, HER2-neu and Ki-67 was not significant.

Keywords: Breast carcinoma, Cluster of differentiation 10, Immunohistochemistry, Prognosis, Stromal marker

INTRODUCTION

Breast cancer is one of the common causes of death due to cancer in women worldwide. According to Indian Council of Medical Research (ICMR), 1.67 million new breast cancer cases were diagnosed in 2012 worldwide. Incidence of breast cancer has overtaken cervical cancer and is the leading cause of cancer death in urban India with an annual incidence of more than 1.5 lakh cases [1]. Invasive breast carcinoma No Special Type (NST) is the most common type of breast carcinoma [2]. The routine immunohistochemical markers used in the evaluation of breast cancer specimens are ER, PR, and HER2-neu. These markers are prognostic indicators and they also decide further clinical management of breast cancer. Many studies have identified that the interaction between stromal components and tumour cells in breast carcinomas is a critical step in tumour progression [3-5]. Tumour stroma plays an important role in tumour invasion and metastasis [6]. Stromal markers have shown to have an important role in assessing the prognosis and treatment of breast carcinoma.

CD10, a cell surface metalloproteinase, which is usually called Common Acute Lymphoblastic Leukemia Antigen (CALLA) was originally described as a cell surface marker for sub classification of acute leukaemias and malignant lymphomas. CD10 has been demonstrated in a variety of normal and neoplastic tissues. This marker has been routinely used in histopathological diagnosis of non haematopoietic tumours [7]. Many studies have been done to find out correlation of stromal CD10 expression with other prognostic markers of breast carcinoma. They have proved an association between stromal CD10 expression and poor prognosis [8-11]. Most of these studies correlated the expression of CD10 with ER, PR and Her2-neu. Only few studies were done to find out association of CD10 with Ki-67.

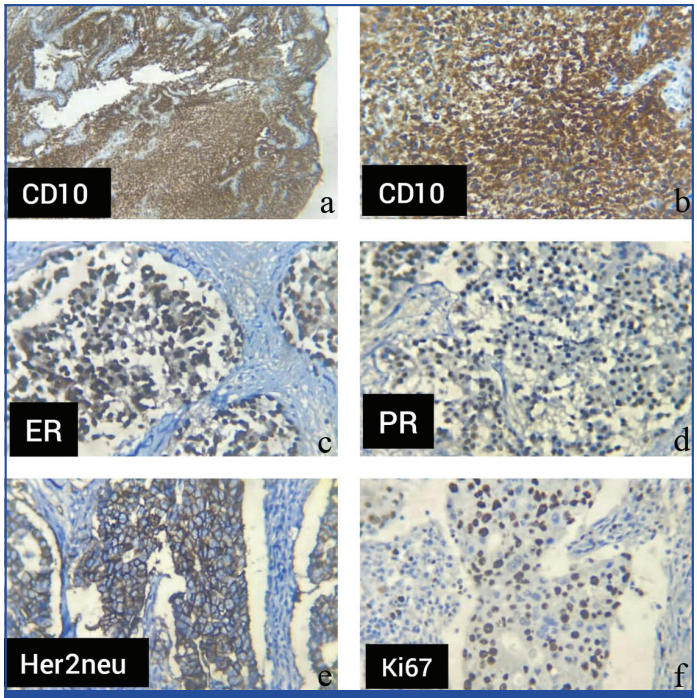
The present study was done to assess the magnitude of stromal expression of CD10 in invasive breast carcinomas and to evaluate prognostic significance of stromal CD10 expression by its association with proven prognostic markers of breast carcinoma like ER, PR, HER2-neu and Ki-67 expression and tumour grade.

MATERIALS AND METHODS

This study was a cross-sectional study in a tertiary care hospital of Government Medical College Kottayam, including mastectomy specimens received in histopathology laboratory during a period of two years from July 2015 to June 2017. Trucut breast biopsies and post chemotherapy mastectomy specimens were excluded from the study. The study was approved by Institutional Review Board (IRB:99/2015).

Formal written informed consent was not availed as it was waived by the institutional Research Committee. Formalin fixed, paraffin-embedded tissue specimens were cut into 5 mm-thick sections, and stained with Haematoxylin and Eosin (H&E). Histopathologic evaluation was done and classification of tumours was done based on World Health Organisation (WHO) classification [2]. Every case of invasive breast carcinoma of no special type during the period of two years was selected which accounted for 50 cases. Nottingham modification of Scarff-bloom-Richardson-Method was utilised for histologic grading of the tumours [12].

Staging was based on the Tumour Node Metastasis (TNM) system adopted by both the Union for International Cancer Control (UICC) and the American Joint Commission on Cancer (AJCC) and was used for staging of tumours [13]. Immunohistochemistry (IHC) was used to assess the expression of ER, PR, HER2-neu, Ki-67 and CD10 [Table/Fig-1]. The immunohistochemistry was done on formalin-fixed paraffin-embedded breast tissue blocks with normal breast tissue serving as positive control for ER and PR. Tissue sections of HER2-neu positive breast cancer were kept as positive control for HER2-neu and myoepithelial cells of normal breast tissue served as positive control for CD10. ER, PR and HER2-neu was reported according to the American Society of Clinical Oncology/College of



[Table/Fig-1]: a) CD10 strong positive cells (10 X); b) CD10 strong positive cells (40); c) ER positive cells (40 X); d) PR positive cells (40 X); e) HER1- neu membrane positivity (40 X); f) Ki-67 positivity cells (40 X).

American Pathologists (ASCO/CAP) Protocols by two Pathologists independently to exclude observation bias [14,15].

When <10% tumour stromal cells showed staining, CD10 immunostaining was considered as negative, weak (either diffuse weak staining or weak or strong focal staining in less that 30% of stromal cells per core) and strong staining (when 30% or more of the stromal cells were present).

Allred scoring system was used to grade ER and PR which is a semi–quantitative system that takes into account the proportion of positive cells (scored on a scale of 0-5) and staining intensity (scored on a scale of 0-3) [16]. The proportion and staining were then added to produce total scores of 0 or 2 through 8. A score of 0-2 was regarded as negative and 3-8 as positive. Tumours having 1% or higher number of cells, nuclear-positive stained for ER and PR are considered positive and if <1% of the tumour cells demonstrated labeling for the hormonal receptors or had an Allred score of 0 to 2, they were labelled as negative.

HER2-neu scoring was done according to the ASCO/CAP guidelines [15]. Score 0 and 1 were considered negative. Score 2 was taken as equivocal which had to be confirmed by Fluorescence In-situ Hybridization (FISH). Hence, HER2-neu 2+ was taken as negative along with HER2-neu 0 and 1+. Only 3+ on IHC was taken as positive in this study. Ki-67 labeling index is the percentage of cells showing Ki-67 positive nuclear immunostaining. A 500 tumour cells were assessed and Ki-67 values were expressed as the percentage of positive cells in each case. Cases showing more than 15% positive nuclei were classified as high Ki-67 expression, and those with less than 15% were classified as low Ki-67 expression [17].

STATISTICAL ANALYSIS

Analysis of data was done performing descriptive statistics using number and percentages as well as inferential statistics with Mantel-Haenzel (MH) Chi-square test. Association between CD10 and ER, PR, HER2-neu, Ki-67 and tumour grade was taken as statistically significant when p-value was less than 0.05.

RESULTS

In this study, all patients were females and mean age of the patient was 57.26 years (range 33-80 years). CD10 immunostaining was done on all 50 cases. Of these, 41 (82%) cases showed CD10 positive and 9 (18%) cases were CD10 negative. Out of these, 41 positive cases, 22 (44%) cases were strong positive and 19 (38%) cases were weak positive [Table/Fig-2]. Both strong and weak CD10 positive cases were evaluated for its association with ER, PR, HER2-neu and Ki-67.

CD10 expression	Number of cases (n)	Percentage (%)
Strong	22	44
Weak	19	38
Negative	9	18

[Table/Fig-2]: CD10 expression in Breast carcinoma (N=50).

Out of 50 cases of breast carcinoma, 31 (62%) were positive for ER. Of the ER +ve cases, 80.6% cases were positive for CD10 and 19.4% cases were negative for CD10. Out of the 19 ER –ve cases, 84.2% cases were positive for CD10 [Table/Fig-3]. Sensitivity of CD10 compared to ER was 80.65% and specificity was 15.68%. Mantel-Haenzel Chi-square test was 0.099 with a p-value of 0.752 which was statistically not significant.

CD10	ER +ve	ER -ve	Total
CD10 +ve	25 (80.6%)	16 (84.2%)	41
CD10 -ve	6 (19.4%)	3 (15.8%)	9
Total	31 (100%)	19 (100%)	50

[Table/Fig-3]: Association of Cluster of Differentiation (CD)10 with ER.
ER: Estrogen recepter; MH: Mantel-Haenzel; Chi-square value 0.099; p-value=0.752

Out of 50 cases, 29 (58%) were PR +ve. Of the 29 PR +ve cases, 24 (83%) were CD10 +ve. Among the 21 PR –ve cases, 17 cases (81%) were CD10 +ve [Table/Fig-4]. Sensitivity of the CD10 positive was found to be 82.76% and specificity 19%. Mantel-Haenzel Chi-square test was 0.026 with a p-value of 0.87 which was statistically not significant.

CD10	PR +ve	PR -ve	Total
CD10 +ve	24 (83%)	17 (81%)	41
CD10 -ve	5 (17%)	4 (19%)	9
Total	29 (100%)	21 (100%)	50

[Table/Fig-4]: Association of CD10 with PR.
PR: Prognostic recepter; MH: Mantel-Haenzel; Chi-square value 0.026; p-value=0.87

Out of 50 cases, 23 (46%) were HER2-neu -ve score 0 or 1, 7 (14%) were HER2-neu +ve score 2 and 20 (40%) were HER2-neu +ve score 3. Of the score 3 HER2-neu +ve cases, 16 (80%) were positive for CD10. Of the 23 HER2-neu -ve cases, 21 (91.3%) were positive for CD10 [Table/Fig-5a]. Seven (14%) HER2-neu +ve cases with score 2 were excluded from the study as these cases were to be confirmed by FISH. Mantel-Haenzel Chi-square test was 1.11 with p-value of 0.292 which was statistically not significant [Table/Fig-5b].

CD10	HER2-neu -ve Score 0 or 1	HER2-neu +ve Score 2	HER2-neu +ve Score 3	Total
Strong	13	3	6	22
Weak	8	1	10	19
Negative	2	3	4	9
Total	23 (46%)	7 (14%)	20 (40%)	50 (100%)

[Table/Fig-5a]: Association of CD10 with HER2-neu.

CD10	HER2-neu +ve	HER2-neu -ve	Total
CD10 +ve	16 (80%)	21 (91.3%)	37
CD10 -ve	4 (20%)	2 (8.7%)	6
Total	20 (100%)	23 (100%)	43

[Table/Fig-5b]: Association of CD10 with HER2-neu. (N=43); Seven (14%) HER2-neu +ve cases with score 2 were excluded from the study as these cases were to be confirmed by FISH; MH: Mantel-Haenzel, Chi-square test value 1.11; p-value 0.292.

Of the 50 cases, 8 (16%) were triple negative cases. Among them, CD10 was positive (strong and weak) in 7 (87.5%) cases [Table/Fig-6]. Of the 45 Ki-67 +ve cases, CD10 was positive in 37 (82.2%) cases [Table/Fig-7] which accounted for sensitivity of 82.2. Mantel-Haenzel Chi-square test was 0.015 with a p-value of 0.903. There was a positive association between CD10 and high Ki-67 but it was not statistically significant.

CD10	Triple negative	Percent (%)
Strong	4	50
Weak	3	37.5
Negative	1	12.5
Total	8	100

[Table/Fig-6]: Association of CD10 with Triple negative cases.

CD10	Ki-67 High	Ki-67 Low	Total
Strong	21 (46.7%)	1 (20%)	22
Weak	16 (35.5%)	3 (60%)	19
Negative	8 (17.8%)	1 (20%)	9
Total	45 (100%)	5 (100%)	50

[Table/Fig-7]: Association of CD10 with Ki 67.
MH: Mantel-Haenzel, Chi-square value 0.015; p-value: 0.903

Of the 32 cases of higher grade tumours, 28 (87.5%) were CD10 positive (strong or weak). Among the 5 lower grade tumours, 2 (40%) showed CD10 positivity [Table/Fig-8]. Mantel-Haenzel Chi-square test was 6.19 with p-value of 0.013 which was statistically significant.

CD10	Tumour Grade 1	Tumour Grade 2	Tumour Grade 3	Total	Percent (%)
Strong	1	12	2	15	40.54
Weak	1	11	3	15	40.54
Negative	3	3	1	7	18.91
Total	5	26	6	37	100

[Table/Fig-8]: Association of CD10 with tumour grade.
MH: Mantel-Haenzel, Chi-square value 6.19; p-value 0.013

DISCUSSION

In the present study, CD10 positive cases accounted for 41 (82%) cases and 9 (18%) cases were CD10 negative. Out of these positive cases 22 (44%) cases were strong positive and 19 (38%) cases were weak positive. This is similar to studies by Rizk AM et al., and Puri V et al., [5,10].

Tumour grade according to Nottingham's grading could be assigned to only 37 cases. Among them, 81.08% were CD10 positive (strong or weak). This indicates that CD10 is a very sensitive test among the graded tumours. Association between CD10 expression and higher grade of tumour was statistically significant (p-value=0.013). Studies by Louhichi T et al., Makretsov NA et al., Puri V et al., and Hosni HN et al., showed a positive correlation between CD10 immunostaining and tumour grade [3,8,10,18]. But the study by Raziq AH and Masoud SM, showed no significant correlation between tumour grade and CD10 immunostaining [9].

There was no significant association between CD10 and ER and PR. Both ER, PR positive and ER, PR negative tumours showed a CD10 strong positivity. Studies by Puri V et al., showed a negative correlation of CD10 with ER and PR. Study by Makretsov NA et al., showed a significant correlation of CD10 with negative ER status but no significant correlation with PR. Sensitivity of CD10 compared to ER was 80.65% and specificity 15.68% [Table/Fig-3]. Though the sensitivity of the test was found to be high, specificity was found to be low. Thus, this can be used as good test for screening purposes but a poor reliable test for excluding the cases.

In the present study, a significant association between CD10 and HER2-neu staining was not seen. In a study by Chattopadhyay M et al., a negative correlation between CD10 and ER and PR status was seen, which was not statistically significant and no correlation was found between CD10 and HER2-neu [19]. Study by Puri V et al., showed a positive correlation of CD10 with HER2-neu but Makretsov NA et al., found that there was no correlation between CD10 and HER2-neu [8,10].

There were eight triple negative cases. CD10 was positive in 87.5% of the cases. This shows a strong association of CD10 positivity with triple negative status but it was not stastically significant. A similar association was found in a study by Kamal M et al., [20]. In their study, the triple negative cases showed a higher CD10 positivity as compared to the non triple negative cases which was statistically not significant. Of the 45 Ki-67 +ve cases, CD10 positivity was seen in 82.2% cases. An association was established between high Ki-67 and CD10 but it was not statistically significant. Positive correlation of CD10 with high Ki-67 was found in studies by Puri V et al., and Chattopadhyay M et al., [10,19]. Only few studies are available correlating CD10 with Ki-67. Lack of significance was attributed to limited number of cases in the study by Chattopadhyay M et al., [19].

Limitation(s)

This study was done including 50 cases of breast carcinoma. More relevant inferences can be made if the study is done in a larger number of cases. FISH was not available for evaluation of score HER2-neu positive cases.

CONCLUSION(S)

In the present study, 82% of breast carcinoma cases showed CD10 positivity. CD10 stromal overexpression positively associated with increasing tumour grade but there was no significant correlation could be obtained between CD10 and ER, PR and HER2-neu status. CD10 stromal overexpression was significantly associated with triple negative cases and high Ki-67. Since, this marker was associated with tumour grade, it can be included in the routine panel of IHC markers as a prognostic marker. It is already proven by various studies that stromal CD10 expression is associated with more aggressive behavior in various epithelial malignancies [7]. Further studies including a larger number of cases and all histopathological types of breast carcinoma are needed to find out the definite role of stromal CD10 expression in targeted therapies.

REFERENCES

[1] Swaminathan S. Consensus document for management of breast cancer. ICMR. 2016:11-12. https://main.icmr.nic.in/sites/default/files/guidelines/consensu_3.pdf.
[2] WHO Classifcation of Tumours Editorial Board, ed. WHO classifcation of tumours, 5th edition- Breast tumours. Lyon: International Agency for Research on Cancer 2019. https://publications.iarc.fr/Book-And-Report-Series/Who-Classification-Of-Tumours.
[3] Louhichi T, Saad H, Dhiab MB, Ziadi S, Trimeche M. Stromal CD10 expression in breast cancer correlates with tumour invasion and cancer stem cell phenotype. BMC Cancer. 2018;18(1):01-09.
[4] Jana SH, Jha BM, Patel C, Jana D, Agarwal A. CD10-a new prognostic stromal marker in breast carcinoma, its utility, limitations and role in breast cancer pathogenesis. Indian J Pathol Microbiol. 2014;57(4):530-36.
[5] Rizk AM, Abdelzaher E, Gowil AG, Elsaka RO. Stromal expression of CD10 in invasive breast carcinoma and its correlation with clinicopathological parameters. Egyptian Journal of Pathology. 2017;37(1):01-07.
[6] Kumar V, Abbas AK, Aster JC, Perkins JA. Robbins Basic Pathology. 10th ed. Philadelphia, PA: Elsevier; 2018.
[7] Yasuda M, Itoh J, Satoh Y, Kumaki N, Tsukinoki K, Ogane N, Osamura RY. Availability of CD10 as a histopathological diagnostic marker. Acta Histochemica et Cytochemica. 2005;38(1):17-24.
[8] Makretsov NA, Hayes M, Carter BA, Dabiri S, Gilks CB, Huntsman DG. Stromal CD10 expression in invasive breast carcinoma correlates with poor prognosis, estrogen receptor negativity, and high grade. Modern Pathology. 2007;20(1):84-89.
[9] Raziq AH, Masoud SM. The correlation of stromal Cd10 expression in invasive breast cancer with various clinicopathological parameters. Iraqi J Comm Med. 2017;3:129-35.
[10] Puri V, Jain M, Thomas S. Stromal expression of CD10 in invasive breast carcinoma and its correlation with ER, PR, HER2-neu and Ki-67. Int J Breast Cancer. 2011;2011:437957-61. Doi: 10.4061/2011/437957. Epub 2011 Jun 16.

[11] Devi HJ, Karkuzhali P, Baskaran PK, Lilly SM. The detailed study of CD10: A stromal marker in breast carcinoma. Journal of Pharmaceutical Research International. 2021:62-70.
[12] Pradhan A, Paudyal P, Sinha AK, Agrawal CS. Grading, staging and Nottingham prognostic index scoring of breast carcinoma. Journal of Pathology of Nepal. 2017;7(1):1078-83.
[13] Amin MB, Edge SB, Greene FL, et al, eds. AJCC Cancer Staging Manual. 8th ed. New York: Springer; 2017.
[14] Hammond ME, Hayes DF, Dowsett M, Allred DC, Hagerty KL, Badve S, et al. American Society of Clinical Oncology/College of American Pathologists guideline recommendations for immunohistochemical testing of estrogen progesterone receptors in breast cancer. Arch Pathol Lab Med. 2010;134(7):e48-72.
[15] Wolff AC, Hammond ME, Hicks DG, Dowsett M, McShane LM, Allison KH, et al. Recommendations for human epidermal growth factor receptor 2 testing in breast cancer: American Society of Clinical Oncology/College of American Pathologists clinical practice guideline update. Archives of Pathology and Laboratory Medicine. 2014;138(2):241-56.
[16] Allred DC, Harvey JM, Berardo M, Clark GM. Prognostic and predictive factors in breast cancer by immunohistochemical analysis. Modern pathology: An official journal of the United States and Canadian Academy of Pathology. Inc. 1998;11(2):155-68.
[17] Soliman NA, Yussif SM. Ki-67 as a prognostic marker according to breast cancer molecular subtype. Cancer Biol Med. 2016;13(4):496-504.
[18] Hosni HN, Abd EL Aziz A, Tabak SA, EL Sayed M. imunohistochemical study of stromal CD10 expression in mammary duct carcinoma. Med J Cairo Univ. 2012;80(2):37-44.
[19] Chattopadhyay M, Giri R, Senapati U. CD10 expression by stromal cells in carcinoma of breast and its correlation with ER, PR, HER2neu and Ki-67-A tissue microarray study in a tertiary care hospital. Indian Journal of Pathology and Oncology. 2019;6(3):445-47.
[20] Kamal M, Khan R, Hasan SH, Maheshwari V. Evaluation of stromal CD10 expression and its correlation with other clinico-pathological factors in invasive breast carcinoma. Indian Journal of Pathology and Oncology. 2019;6(3):417-21.

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