ISSN: 2581 – 3633
PubMed - National Library of Medicine - ID: 101745081

International Journal of Medical Science and Advanced Clinical Research (IJMACR)

Available Online at:www.ijmacr.com

Volume – 6, Issue – 3, May- 2023, Page No. : 158 - 166

Immunohistochemical Stromal Expression of CD10 In Invasive Ductal Carcinoma Breast and Its Correlation with Histological Grade

[1]Mayank Jain, Senior Resident, Department of Pathology, BRD Medical College, Gorakhpur, Uttar Pradesh, India

[3]Shaila Mitra, Professor & HOD, Department of Pathology, BRD Medical College, Gorakhpur, Uttar Pradesh, India

[3]Rajesh Rai, Professor, Department of Pathology, BRD Medical College, Gorakhpur, Uttar Pradesh, India

[4]Yogesh Kumar, Professor & HOD, Department of Surgery, BRD Medical College, Gorakhpur, Uttar Pradesh, India

[5]Shubham Mishra, Junior Resident, Department of Pathology, BRD Medical College, Gorakhpur, Uttar Pradesh, India

[6]Dr Pallavi Singh Sabal, Junior Resident , Dept of Pathology, BRD Medical College, Gorakhpur, Uttar Pradesh, India

Corresponding Author: Mayank Jain, Senior Resident, Department of Pathology, BRD Medical College, Gorakhpur, Uttar Pradesh, India.

How to citation this article: Mayank Jain, Shaila Mitra, Rajesh Rai, Yogesh Kumar, Shubham Mishra, Dr Pallavi Singh Sabal, "Immunohistochemical Stromal Expression of CD10 In Invasive Ductal Carcinoma Breast and Its Correlation with Histological Grade", IJMACR- May- 2023, Volume – 6, Issue - 3, P. No. 158 – 166.

Type of Publication: Original Research Article

Conflicts of Interest: Nil

Abstract

CD10 is a cell surface zinc-dependent endopeptidase, which degrades many bioactive peptides. Multiple studies in past have associated CD 10 expression in tumor stroma with aggressiveness of many epithelial malignancies, but use of this marker as a clinically significant tumor marker for diagnosis and prognosis is still in quandary. The aim of this study was to evaluate the expression of CD10 in breast cancer and its correlation with histopathological grading of breast carcinomas.

A total of 110 cases of formalin fixed paraffin embedded histological sections of various breast masses including intraductal and invasive ductal carcinoma in period of one year were assembled. Stromal CD 10 expression was assessed by immunohistochemistry and scored as negative, weak and strong. Stromal CD10 positivity was seen in 76.2% of malignant cases, out of which strong positivity was observed in 42.9 % cases. It was preferentially expressed in invasive compared to non-invasive breast cancers ($p < 0.00001$).

A strong correlation was observed between stromal CD10 expression and increasing tumor grade ($p=0.00137$).

Conclusion: Stromal expression of CD10 in invasive carcinoma of breast is associated with high tumor grade and can be developed as potential independent

prognostic marker, as well as a potent target for development of novel therapies.

Keywords: CD10, Breast cancer, tumour.

Introduction

Breast cancer is the most common cancer among women in India according to National cancer registry programme 2011 report.[1] Worldwide it is the most common non-skin cancer in females.[2] By the year 2030 global burden of breast cancer will be more than two million every year.[3] At present the mortality rate for breast cancer in India is 11.1per 10,000.[4] Breast tissue is composed of duct (epithelial origin) and stroma (mesenchymal origin). Epithelial growth of tumour depends partly on chemical mediators between tumour cells and stromal cells.[5] Although breast cancer is an epithelial malignancy, stroma plays a key role in modulating tumor invasion and metstasis. A better understanding of stromal contribution to cancer progression will identify specific signals that promote growth, dedifferentiation, invasion, and ectopic survival of tumor cells and may eventually result in the identification of new therapeutic targets for future treatment.[6]

CD 10 a stromal marker is an emerging novel prognostic marker, showing correlation with the grading of invasive carcinoma. It is a myoepithelial marker.[7]

CD 10 is a zinc dependent metalloproteinase that has been called common acute lymphoblastic antigen (CALLA). It is frequently expressed in bone marrow lymphoid stem cells, pro-B lymphoblasts, mature neutrophils, various lymphoma subtypes, renal cell carcinoma, and endometrial stromal sarcoma. Several reports indicated that stromal CD10 expression is associated with biological aggressiveness in various epithelial malignancies.[5,8-11]

Aims and Objectives

- To analyse the stromal expression of CD10 in invasive breast carcinoma and compare it with adjacent normal breast parenchyma and benign neoplastic lesions.
- To correlate the stromal expression of CD10 with histopathological grading of breast carcinomas.

Materials and Methods:

A prospective study was conducted on 110 Modified radical mastectomy specimens which were sent to Department of Pathology, B.R.D Medical College Gorakhpur from July 2019 to June 2020. Relevant history like age , menopausal status, duration, previous treatment was taken.

All specimens were formalin fixed, representative sections were taken and H &E staining was done. The grading was done according to Nottingham's combined histologic grade (Elston-Ellis modification of Scarff - Bloom –Richardson grading system)

Immunohistochemistry for CD10

5µ sections were taken on poly vinyl chloride coated slides. Sections were deparaffinised in xylene followed by hydration in descending ethanol grades. Antigen retrieval was done by microwave procedure.

Slides were put in a container having citrate buffer and kept it in the microwave at 80^{o} C for 5 minutes and 2 such changes were done. Following this slides were kept in microwave at 100^{o} C for 5 minutes. Slides were brought to room temperature and washed with PBS. Peroxidase block was added and washing with PBS was done. Power block was added on the sections and incubation at room temperature in humidity chamber was done for 60 min. Sections were again washed with PBS and super enhancer was added to sections with incubation at room temp for 30 min. Sections were

washed with PBS as before and Polymer HRP (secondary antibody) was added on sections and incubated in humidity chamber for 7 min was done. Sections were taken out and washed with distilled water and counterstained with Hematoxylin for 30 secs. Finally sections were brought to running water for 5 min and dehydration was performed with ascending grades of alcohol. Sections were air dried for 20 min and mounted in DPX.

CD 10 Staining	**Score**	**Result**
No Staining	0	Negative
Focal or diffuse weak staining, Strong focal staining < 30 %	1	Weak
Strong staining ≥ 30% stromal positive cells	2	Strong

Table 1: CD 10 Scoring

Statistical analysis was performed by using Graph pad software. The correlation between stromal cells CD10 expression and clinicopathological features was evaluated using the chi-square test. A p-value < 0.05 was considered as statistically significant.

Results

Out of 110 cases in our study population, 68 cases were of benign breast lesion while 42 were malignant. In benign breast lesions, out of 68 cases, 61 (89.7%) were negative for stromal CD10 expression. Weak expression was observed in 6 (8.8%) cases. CD10 expression in fibroadenoma was limited to myoepithelium, only 2 out of 51 cases of fibroadenoma showed weak stromal CD10 expression. Weak and patchy CD10 staining was seen in 2 of 3 benign phyllodes cases. One case of fibrocystic breast lesion and both cases of atypical ductal hyperplasia also demonstrated weak CD10 stromal expression. No stromal expression was detected in the adjacent normal breast tissue. The myoepithelial cells lining the normal acinar and ductal structure showed CD10 expression, however, there was no expression of CD10 in normal ductal cells, fibroblasts and adipose cells. In malignant lesions 76.2% (32 out of 42) of cases showed positivity for CD10 in the stroma, of which 47.7% (20 out of 42) were found to be strongly positive for stromal CD10 staining whereas 12 cases (28.6%) were reported to be weakly positive. 10 cases (23.8%) were negative for CD10 staining.

Statistical significant association was seen between stromal expression of CD10 in malignant breast lesions when compared to benign breast lesions. ($p<0.05$)

Table2. Stromal expression of CD10 in benign and malignant breast lesions

Stromal CD10 Expression	Negative No (%)	Weak Positive No (%)	Strong Positive No (%)
MALIGNANT n=42	10 (23.8%)	14 (33.3%)	18 (42.9%)
BENIGN n=68	61 (89.7%)	06 (8.8%)	01 (1.5%)

p-value<0.00001

Fig.1: Stromal expression of cd10 in breast lesions

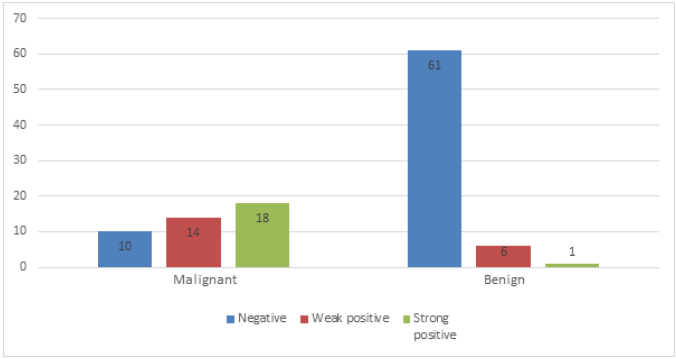
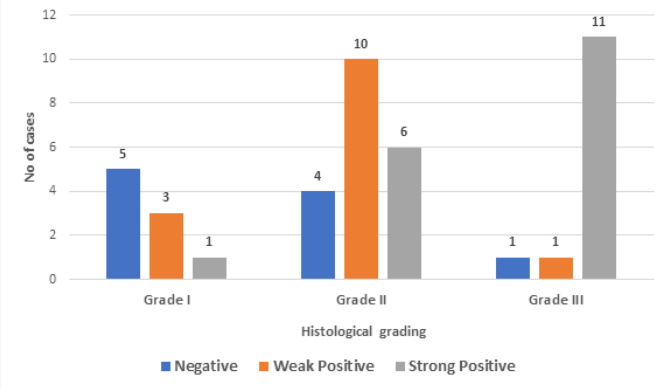


Table 3: Stromal expression of cd10 malignant breast lesions & histological grading

Grade	Negative	Weak positive	Strong positive	Total	Chi^2	'p' value
Grade	05	03	01	09		

i	(55.6%)	(33.3%)	(11.1%)			
Grade ii	04 (20.0%)	10 (50.0%)	06 (30.0%)	20	17.7577	0.00137
Grade iii	01 (7.7%)	01 (7.7%)	11 (84.6%)	13		
Total	10	14	18	42		

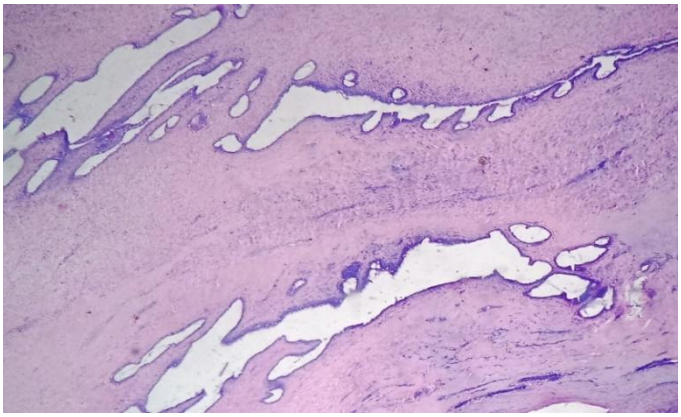
Fig. 2: Stromal expression of cd10 in malignant breast lesions in correlation with histological grading



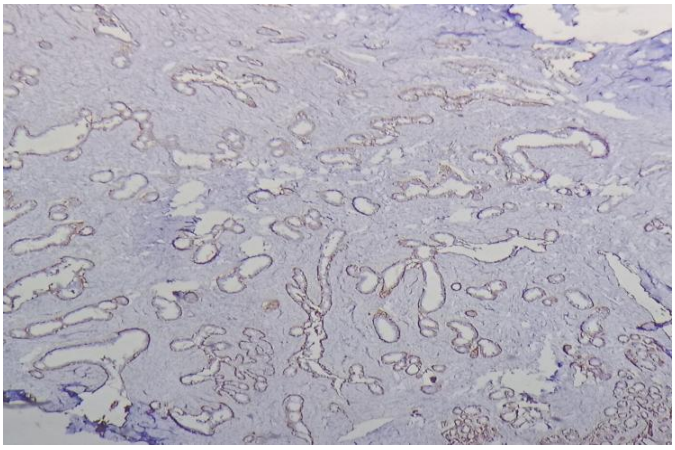
Study of stromal expression of CD10 in breast carcinoma and association with histological grading is shown in Table 3 and Fig 2 reveals ,out of 13 cases belonging to Grade III , 11 cases (84.6%) expressed strong positivity for CD10 and of rest 2 ,one case (7.7%) was weakly positive while other was negative for CD10.

In 20 cases of grade II ,10 (50.0 %) and 4(20.0%) cases respectively were weakly positive and negative for CD10, while 6 (30.0%) cases demonstrated strong positivity for CD10. Of 9 cases in Grade I , 5 (55.6%) were negative for CD10 ,3 (33.3%) stained weakly positive and only 1 case (11.1%) was strongly positive for CD10. Thus it is observed that with increasing grade of tumor, positivity for stromal CD10 expression also increases.

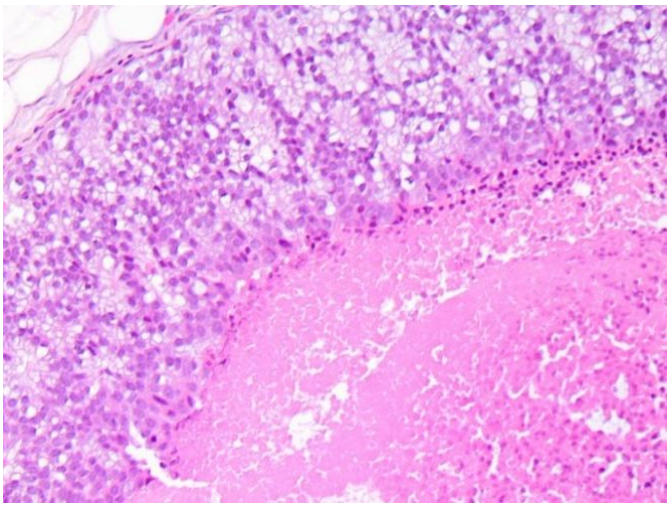
The association between them was found to be statistically significant with p-value being 0.00137 ($p<0.05$)



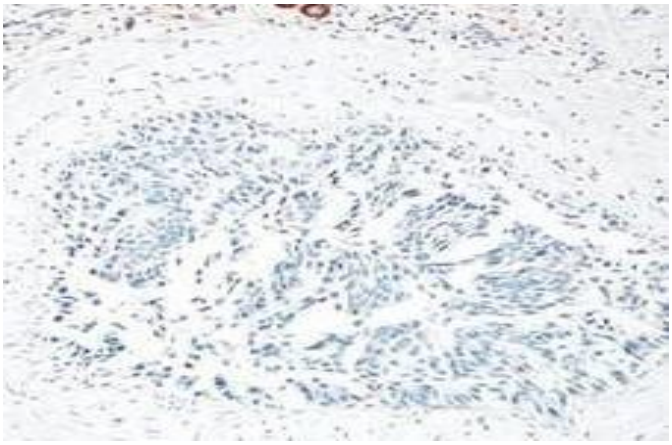
Microphotograph1: H&E stained section of fibroadenoma showing glandular and stromal proliferation (Pericanalicular pattern) (X100)



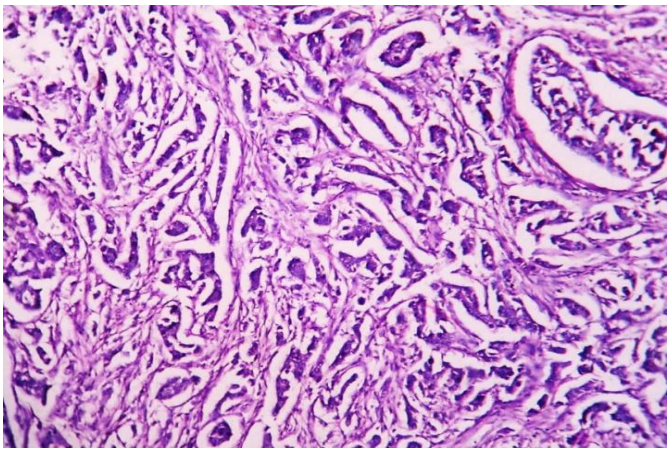
Microphotograph 2: Immunohistochemical staining of CD10 in fibroadenoma (Pericanalicular pattern) showing myoepithelial membranous positivity. (X100)



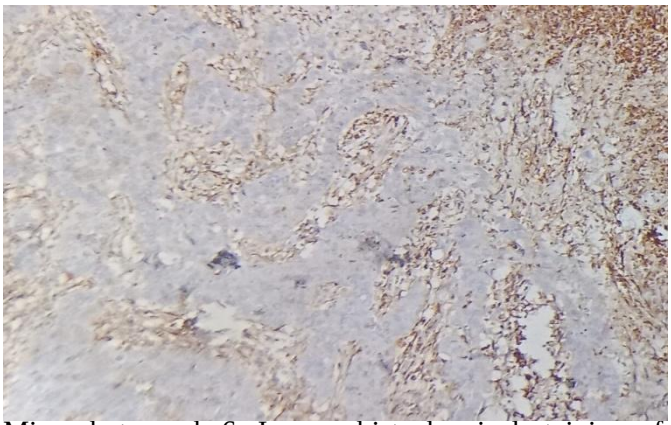
Microphotograph 3: H&E stained section of intermediate grade ductal carcinoma in situ. (X400)



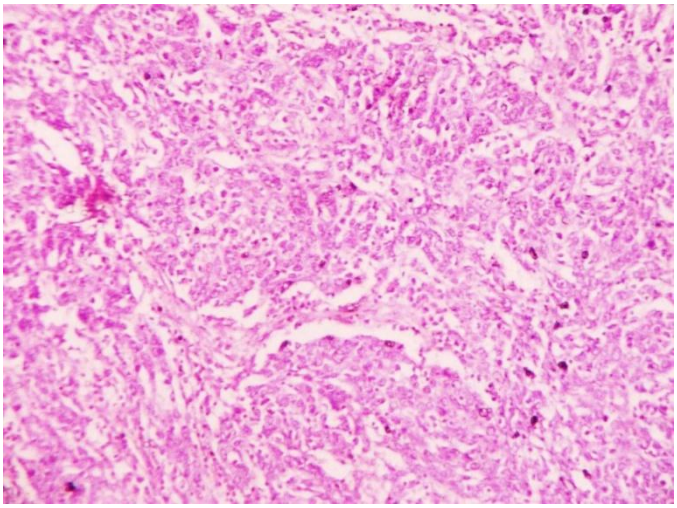
Microphotograph 4: Immunohistochemical staining of CD10 in ductal carcinoma in situ showing weak membranous positivity. (400X)



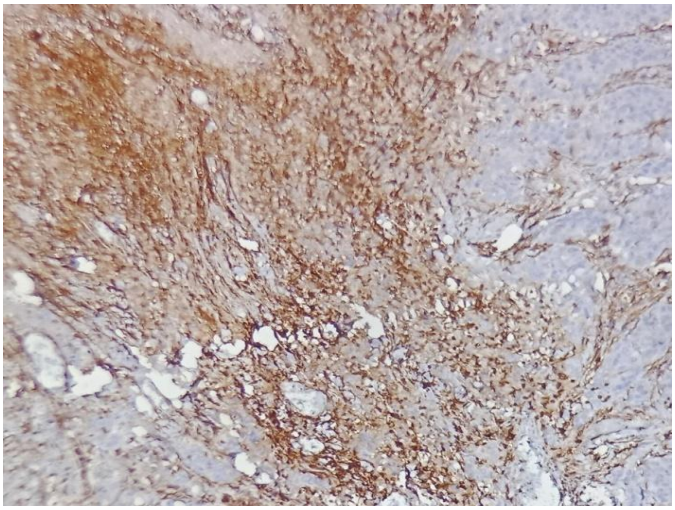
Microphotograph 5:H&E stained section of invasive ductal carcinoma showing tubules and solid trabeculae. (Grade I) The nuclei show relatively less pleomorphism and rate of mitotic activity is low. (400X)



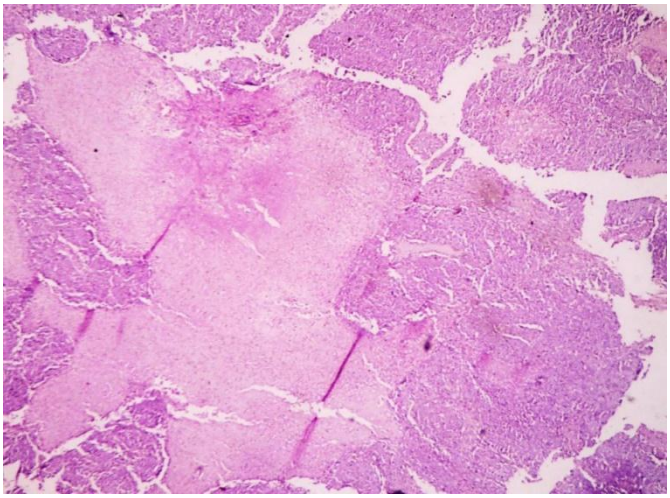
Microphotograph 6: Immunohistochemical staining of invasive ductal carcinoma showing weak stromal positivity for CD10. (400X)



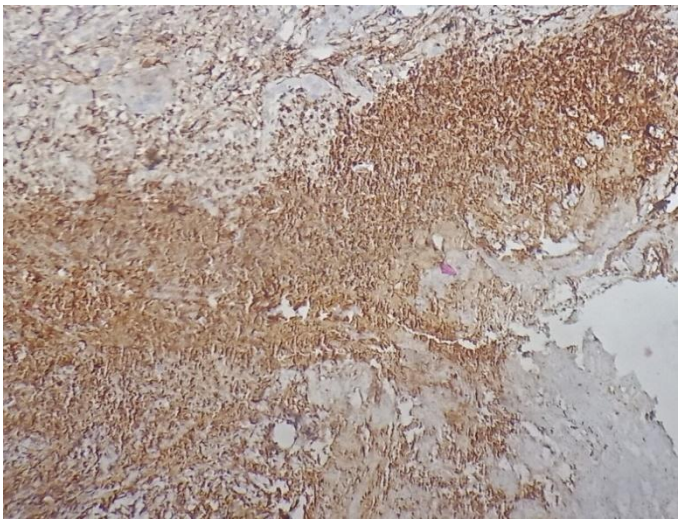
Microphotograph 7: H&E stained section of invasive ductal carcinoma (Grade II) Individual tumor cell shows pleomorphism, mitotic activity, hyperchromasia and prominent nucleoli. (400X)



Microphotograph 8: Immunohistochemical staining of invasive ductal carcinoma showing weak stromal positivity for CD10. (400X)



Microphotograph 9: H&E stained section of invasive ductal carcinoma Grade III showing sheets of dysplastic cells. (X100)



Microphotograph 10: Immunohistochemical staining of invasive ductal carcinoma showing strong stromal positivity for CD10. (X100)

Discussion

Recently, it is well documented that the interaction of cancer cells with their microenvironmentpromotes tumor progression.[12] This interaction involves several factors that influence signaling pathways related to tumor invasion and metastatic dissemination.[13]

Although breast cancer is an epithelial malignancy arising in the epithelial cells of the terminal ductal lobular unit, stromal microenvironment plays an important role in breast cancer evolution and metastasis.[14]The interaction between normal epithelial cells and stromal cells is modified by severalfactors secreted by the tumor cells themselves or by stromal cells under the influence of tumor cells.[14] One such important factor is the matrix metalloproteinase (MMP). MMP plays an important role in tumor progression as well as defining the role of stromal microenvironment in tumor invasion and metastasis.[15] CD10 is a cell surface zinc-dependent metalloproteinase that cleaves the protein components of extracellular matrix and thereby plays a pivotal role in tissue remodeling.[16]

The findings of our studies are recapitulated and compared with the results of other authors, indicating agreement or contrast with previously published work and pointing out exceptions and lack of correlation.

In a study done by **Makretsov et al**,[5] 79% (205 out of 258) of invasive ductal carcinoma of breast showed stromal CD10 expression, **Balaji T et al**[17]observed positivity in 73% of cases of which 46% (14 cases) was strongly positive and 27% were weakly positive**. Puri V et al**[16] also found CD10 expression in 80% (40 out of 50 cases) of invasive ductal carcinoma of breast. Study done by **Thomas S et al**[17] showed stromal CD10 positivity in 55% (16 out of 29 cases).

In our study, stromal CD10 positivity was reported in 76.2% (32 out of 42) of malignant cases, of which 47.7% (20 out of 42) were found to be strongly positive for stromal CD10 staining whereas 12 cases (28.6%) were reported to be weakly positive. 10 cases (23.8%) were negative for CD10 staining.

Several studies such as **Dhande N et al,**[19] **Jana S et al,**[14]**Rizk A M et al,** [20] **Anuradha B V et al**[21] **and Louhichi T et al**[22] corroborated the similar finding of stromal expression of CD10 in malignant breast lesions.

However, **Iwaya etal**[9] reported in their study on 110 malignant cases, only 18% cases were positive for stromal CD10 expression.

Similarly, **Kermani et al**[23] reported a significant correlation between stromal positivity for CD10 and histological grading. Out of 22 grade I tumors, majority (68.2%, 15 cases) did not show stromal CD10 expression, where as 53.6% of Grade III tumors were strongly positive for CD10. 20 cases (40%) belonging to grade II expressed weak positivity for stromal CD10.

A statistically significant association was reported between stromal CD10 immunoexpression and tumor grade. ($p = 0.00137$) in our study. 84.6% cases of grade III expressed strong stromal positivity for CD10, whereas 55.6% cases were negative for stromal CD10 expression.

This results in agreement with those reported by other authors (**Rizk A et al,**[20] **Balaji T et al,**[17]**Marketsov et al**[5]**, Anuradha B V et al,**[21]**Sadaka E et al,**[24]**KermaniM et al**[23]**, Dhande N et al**[19]**, Premlatha S et al,**[25]**Louhichi T et al**[22]**, Jana S et al**[14])

Thus, a stronger CD10 expression in a higher tumor grade may suggest a role of CD10in tumor differentiation and aggressiveness.

In contrast to our findings**, Iwaya et al,**[9] **Puri et al**[16] **and Vo Diem et al**[26] reported that there was no statistically significant correlation between CD10 expression and different tumor grades. The lack of standardized methodology for measuring stromal CD10 expression and the use of different cut off points might explain these different findings.

From the present study, it can be suggested that stromal CD10 expression may be implicated in breast cancer tumorigenesis. Expression of CD10 was observed more in malignant cases as compared to benign lesions. It was also found to be associated with high tumor grade which also suggest that CD10 may contribute to tumor aggressiveness and progression. Strong positive CD10 expression was significantly associated with increasing tumor grade. As the tumor grade increases, stromal CD10 expression is increased.

However, these results need to be supported by further studies with larger sample sizes to further elucidate the impact of CD10 on breast cancer patients' outcome.

Conclusion

Stromal expression of CD10 was significantly associated with higher tumor grade indicating that CD10 can be used as an independent prognostic marker and should be included in routine histopathology report. CD10 could act as a potential target for newer drug development.

References

1. NCRP, Three year report of population based cancer registries. 2009-2011, ICMR.
2. Robins and Cotran Pathologic Basis of Disease. 9th ed. volume II. Elsevier; 2015:1051.
3. Ferlay J, Soerjomataram I, Ervik M, Dikshit R, Eser S, Mathers C, et al. Cancer Incidence and Mortality Worldwide: IARC Cancer Base No.11.
4. Surakasula A, Nagarjunapu GC, Raghavaiah KV. A comparative study of pre- and post-menopausal breast cancer: Risk factors, presentation, characteristics and management. J Res Pharm Pract. 2014;3:12-8.
5. Makretsov NA, Hayes M, Carter BA et al. Stromal CD10 expression in invasive breast carcinoma correlates with poor prognosis, estrogen receptor negativity, and high grade, Modern Pathology. 2007;20:84-9
6. Weaver O.D., Mareel M,, "Role of tissue stroma in cancer cell invasion," Journal of Pathology, vol. 200, no. 4, pp. 429–447, 2003.

7. Kalof AN, Tam D, Beatty B, Cooper K. Immunostaining patterns of myoepithelial cells in breast lesions: a comparison of CD10 and smooth muscle myosin heavy chain J Clin Pathol. 2004;57:625-9.
8. Huang W.B., Zhou X.J., Chen J.Y. et al., "CD10-positive stromal cells in gastric carcinoma: correlation with invasion and metastasis," Japanese J. Clinical Oncol., vol. 35, no. 5, pp. 245–250, 2005.
9. Iwaya K, H. Ogawa H., M. Izumi M. et al, "Stromal expression of CD10 in invasive breast carcinoma: a new predictor of clinical outcome," VirchowsArchiv, vol. 440, no. 6, pp. 589–593, 2002.
10. Tokuhara T., Adachi M., Hashida H. et al., "Neutral endopeptidase/CD10 and aminopeptidase N/CD13 gene expression as a prognostic factor in non-small cell lung cancer," Japanese J of Thoracic and Cardiovascular Surgery, vol. 49, no. 8, pp. 489–496, 2001.
11. Tse G.M.K, Tsang A.K.H, Putti T.C et al., "Stromal CD10 expression in mammary fibroadenomas and phyllodes tumours," J. Clinical Pathol, vol. 58, no. 2, pp. 185– 189, 2005.
12. Kitamura H, Okudela K, Yazawa T, Sato H, Shimoyamada H. Cancer stem cell: implications in cancer biology and therapy with special reference to lung cancer. Lung Cancer Amst Neth. 2009;66:275–281.
13. Kesse-Adu R, Shousha S. Myoepithelial markers are expressed in at least 29% of oestrogen receptor negative invasise breast carcinoma. Mod Pathol. 2004;17:646–652.
14. 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 PatholMicrobiol. 2014;57:530–6.
15. Curran CS, Keely PJ. Breast tumor and stromal cell responses to TGF-β and hypoxia in matrix deposition. Matrix Biol. 2013;32:95–105.
16. Puri V, Jain M, Thomas S. Stromal expression of CD10 in invasive breast carcinoma and its correlation with ER, PR, HER2-neu, and Ki67. Int J Breast Cancer. 2011;2011:437957.
17. Balajji TGS, Ulaganathan S, Venkatachalam AS et al. An analysis of stromal expression of CD10 in invasive ductal carcinoma of breast and its correlation with histological grade. Int J Res Med Sci 2017;5:1629-35
18. Thomas S, Babu RJ, Agarwal K et al. Effect of neoadjuvant chemotherapy on stromal CD10 antigens in breast cancer- A preliminary study. Indian J Cancer. 2013;50:46-51.
19. Dhande A N, Gaurish S, Khandeparkar S et al. Stromal expression of CD10 in breast carcinoma and its correlation with clinicopathological parameters. South Asian J Cancer. 2019 Jan-Mar; 8(1): 18–21.
20. Rizk A M, AbdelzaheraE ,Gowil A G et al . Stromal expression of CD10 in invasive breast carcinoma and its correlation with clinicopathological parameters, Egypt J Pathol 2017, 37:1–7
21. Anuradha BV, S. Chandra Sekhar S.,Saritha C et al. A study on stromal CD10 expression in invasive breast carcinoma. IAIM, 2016; 3(6): 142-147.
22. Louhichi T, Saad H, Dhiab M B et al. Stromal CD10 expression in breast cancer correlates with tumor invasion and cancer stem cell phenotype, BMC Cancer. 2018; 18: 49.
23. Kermani A T, Jafarian A H ,Ashabyamin R et al. The Stromal Overexpression of CD10 in Invasive

Breast Cancer and its Association with Clincophathologic Factors. Iran J Cancer Prev. 2014; 7(1): 17–21.

24. Sadaka E, Almorsy W, Elsaka A. CD10 Expression as a Prognostic Factor in Female Patients with Invasive Ductal Carcinoma of the Breast. J Am Sci 2016;12(4):71-77.
25. Premlatha S et al. Analysis of CD10 expression in breast carcinoma in post menopausal patients. IOSR J Dent &Med.Scn 16.7 (2017):71-74
26. Vo Diem et al. Prognostic impact of CD10 expression in clinical outcome of invasive breast carcinoma. Breast Cancer (2015) 22:117–128.