

Original Research Article

HISTOMORPHOLOGICAL EVALUATION OF BREAST CARCINOMA WITH SPECIAL REFERENCE TO CD10 EXPRESSION IN TUMOUR STROMA AND ITS CLINICOPATHOLOGICAL CORRELATION

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ABSTRACT

Background: Stromal CD10 expression was correlated with clinicopathological and molecular parameters to determine its association with established prognostic factors and to evaluate its potential utility as a prognostic biomarker in breast carcinoma. The aim and objective is to evaluate the histomorphological characteristics of various types of breast carcinoma and analyse the immunohistochemical expression patterns of ER, PR, HER2/neu, and CD10, with special emphasis on the association of stromal CD10 expression with tumour grade and prognostic factors including age, tumour size, histological type, lymphovascular invasion, lymph node status, and hormonal receptor and HER2/neu status.

Materials and Methods: This prospective study was conducted from July 2018 to July 2020 in the Department of Pathology, Guntur Medical College and Government General Hospital, Guntur. A total of 102 histologically confirmed cases of breast carcinoma were included. Formalin-fixed, paraffin-embedded tissue sections were processed routinely and stained with hematoxylin and eosin for histopathological evaluation. Immunohistochemistry for estrogen receptor (ER), progesterone receptor (PR), HER2/neu, and stromal CD10 was performed using the streptavidin-biotin-peroxidase method. Stromal CD10 expression was evaluated and correlated with clinicopathological and molecular prognostic parameters using appropriate statistical analysis.

Results: Among 302 breast specimens, 102 (33.77%) were diagnosed as breast carcinoma. Grade II tumors and invasive carcinoma NST were the predominant findings. Strong stromal CD10 expression was significantly associated with higher histological grade, ER negativity, PR negativity, and HER2 positivity ($p < 0.05$). These findings suggest that stromal CD10 is a useful marker of aggressive tumor behavior and poor prognosis.

Conclusion: Stromal CD10 expression was significantly associated with higher histological grade, ER negativity, PR negativity, and HER2/neu positivity, indicating its association with aggressive breast carcinoma and poor prognosis. Stromal CD10 may serve as a simple, cost-effective prognostic biomarker and could be incorporated into routine histopathological evaluation for improved prognostic assessment.

Keywords: Histomorphological study of breast carcinoma, CD10 stromal marker, Carcinoma, Breast cancer.

INTRODUCTION

Breast carcinoma is one of the most common malignancies affecting women worldwide and

remains a significant public health challenge due to its high incidence and cancer-related mortality. The burden of breast cancer has increased substantially in developing countries, including India, owing to

changing lifestyle patterns, delayed presentation, and variations in access to screening and treatment facilities. Although advances in diagnostic techniques and therapeutic strategies have improved patient outcomes, breast carcinoma continues to demonstrate considerable biological heterogeneity, with differences in clinical behaviour, treatment response, and prognosis among patients. Therefore, identification of additional biomarkers that can provide insight into tumour aggressiveness and progression remains an important area of research. Histopathological evaluation continues to be the cornerstone for diagnosis and prognostic assessment of breast carcinoma. Parameters such as tumour type, histological grade, tumour size, lymph node involvement, and lymphovascular invasion provide essential information regarding disease behaviour. The Nottingham histological grading system, based on tubule formation, nuclear pleomorphism, and mitotic activity, is widely accepted for assessing tumour differentiation and predicting prognosis. However, conventional morphological parameters alone may not completely explain the diverse biological behaviour observed among breast cancer patients. This has resulted in increasing interest in studying the tumour microenvironment and its contribution to cancer progression.^[1] The tumour microenvironment consists of various cellular and non-cellular components, including fibroblasts, immune cells, endothelial cells, extracellular matrix proteins, and signalling molecules. These stromal components actively participate in tumour initiation, invasion, metastasis, and therapeutic resistance rather than acting merely as passive supporting structures. Tumour-associated stromal cells communicate with malignant epithelial cells through complex molecular pathways, resulting in changes that promote tumour growth and progression. Therefore, evaluation of stromal markers has emerged as an important approach for understanding tumour biology and identifying potential prognostic indicators.^[2] CD10, also known as neutral endopeptidase or neprilysin, is a cell surface zinc-dependent metalloproteinase involved in the degradation of several biologically active peptides. Although CD10 expression is well recognised in various normal tissues and haematolymphoid malignancies, its expression in solid tumours has gained attention because of its potential role in tumour invasion and metastasis. In breast carcinoma, CD10 may be expressed by tumour-associated stromal cells, particularly myofibroblasts present around invasive tumour nests. These stromal cells may influence tumour progression by modifying the extracellular matrix and altering communication between cancer cells and surrounding tissues.^[3] Studies have demonstrated that stromal CD10 expression in breast carcinoma may be associated with aggressive tumour characteristics, including higher histological grade, increased invasiveness, lymph node metastasis, and poor prognostic features. Makretsov et al. reported that stromal CD10

expression was associated with adverse pathological parameters and suggested its potential role as a prognostic biomarker in invasive breast carcinoma.^[4] Similarly, Iwaya et al. observed that CD10-positive stromal cells were more frequently associated with invasive and biologically aggressive breast tumours, supporting the importance of tumour–stroma interactions in cancer progression.^[5] The molecular classification of breast carcinoma into different subtypes based on hormone receptor status and HER2 expression has further improved understanding of tumour behaviour. However, additional markers reflecting stromal alterations may provide complementary prognostic information. CD10 evaluation may help identify variations in tumour biology that are not completely captured by conventional immunohistochemical markers such as estrogen receptor, progesterone receptor, and HER2. Several investigators have explored the relationship between stromal CD10 expression and clinicopathological characteristics, although findings have shown variations among different populations.^[6-8] In the Indian context, breast carcinoma often presents at a relatively advanced stage, highlighting the need for easily applicable and cost-effective prognostic markers. Immunohistochemical assessment of stromal CD10 expression can be performed on routinely processed paraffin-embedded tissue specimens and may provide additional information without requiring sophisticated molecular techniques. Understanding the significance of CD10-positive stromal cells may contribute to better risk stratification and improved prognostic assessment of breast carcinoma patients.^[9] The present study aims to evaluate the histomorphological features of breast carcinoma and assess CD10 expression in tumour stroma using immunohistochemistry. The expression pattern of CD10 will be correlated with important clinicopathological parameters such as tumour type, histological grade, tumour size, lymph node status, and other available prognostic factors. This study may provide further insight into the role of tumour stroma in breast carcinoma progression and the potential utility of CD10 as a prognostic biomarker.^[10]

Aim and objectives: To evaluate the histomorphological characteristics of various types of breast carcinoma and analyse the immunohistochemical expression patterns of ER, PR, HER2/neu, and CD10, with special emphasis on the association of stromal CD10 expression with tumour grade and prognostic factors including age, tumour size, histological type, lymphovascular invasion, lymph node status, and hormonal receptor and HER2/neu status.

MATERIALS AND METHODS

This prospective study was conducted from July 2018 to July 2020 in the Department of Pathology, Guntur

Medical College and its associated Government General Hospital. A total of 102 histologically confirmed cases of breast carcinoma were included in the study. Representative tissue specimens received in the Department of Pathology were subjected to routine histopathological processing and immunohistochemical evaluation. The findings were subsequently analysed and correlated with various clinicopathological and prognostic parameters. All tissue specimens were fixed in 10% neutral buffered formalin overnight and processed by conventional paraffin embedding techniques. The fixed tissues were subjected to sequential dehydration using graded concentrations of alcohol (50%, 70%, 90%, and absolute alcohol), followed by clearing with xylene. The tissues were then impregnated with molten paraffin wax in two changes, embedded to prepare paraffin blocks, and sectioned. Sections were stained routinely with haematoxylin and eosin (H&E) for histopathological evaluation. H&E staining was performed after deparaffinization, rehydration, nuclear staining with haematoxylin, differentiation, eosin counterstaining, dehydration, clearing, and mounting with DPX. On microscopic examination, nuclei appeared blue, while the cytoplasm demonstrated varying shades of eosinophilic pink staining. Histopathological evaluation was performed to determine the tumour type, morphological features, and histological grade according to standard classification criteria. The association of histological findings with clinicopathological parameters was assessed. Immunohistochemical analysis was performed for estrogen receptor (ER), progesterone receptor (PR), HER2/neu, and CD10 using the streptavidin–biotin–peroxidase complex method with commercially available Dako reagents. Antigen retrieval was performed using microwave heating. Endogenous peroxidase activity was blocked using 3% hydrogen peroxide, followed by incubation with primary antibodies and appropriate secondary antibodies. Immunoreactivity was visualized using

3,3'-diaminobenzidine (DAB) chromogen, followed by counterstaining with Harris haematoxylin and mounting. CD10 expression was evaluated specifically in tumour-associated stromal cells, and adjacent normal breast parenchyma served as an internal positive control. The intensity and distribution of immunostaining were assessed using established scoring criteria. The study included cases of invasive breast carcinoma from patients of both sexes and all age groups. Cases diagnosed as benign breast lesions, inflammatory breast lesions, and non-epithelial malignancies were excluded from the study. The immunohistochemical expression patterns of ER, PR, HER2/neu, and CD10 were correlated with tumour grade and prognostic parameters including age, tumour size, histological subtype, lymphovascular invasion, and lymph node status. Statistical analysis was performed to determine the significance of associations between CD10 expression and clinicopathological characteristics.

Statistical analysis: The collected data were entered and analysed using SPSS version 25.0 software. Categorical variables were expressed as frequencies and percentages. The histopathological parameters, including tumour type, tumour differentiation, histological grade, and other clinicopathological characteristics, were evaluated and correlated with the immunohistochemical expression of stromal CD10 in breast carcinoma. The association between CD10 expression and various histological parameters was assessed using appropriate statistical methods. Analysis of variance (ANOVA) was applied to determine the differences and association between CD10 expression patterns and tumour differentiation. The statistical significance of the observed associations was determined by calculating the p-value, with a value of <0.05 considered statistically significant. All statistical analyses were performed assuming the standard conditions and assumptions applicable to the respective statistical tests.

RESULTS

Table 1: Clinicopathological profile of breast carcinoma cases (n = 102)

Variable	Category	No. of cases	Percentage (%)
Age (years)	21–40	19	18.62
	41–50	36	35.29
	51–60	25	24.50
	>60	22	21.58
Tumor size	<2 cm	1	0.9
	2–5 cm	71	69.7
	>5 cm	30	29.4
Histological grade	Grade I	17	16.66
	Grade II	43	42.15
	Grade III	42	41.17
Histological type	Invasive carcinoma NST	77	75.49
	NST with Paget disease	3	2.94
	Mucinous carcinoma	9	8.82
	NST with medullary features	9	8.82
	Metaplastic carcinoma	2	1.96
	Cribiform carcinoma	1	0.98
	Adenoid cystic carcinoma	1	0.98
Lymphovascular invasion	Present	57	55.89

	Absent	45	44.11
Lymph node status	N0	37	36.3
	N1	25	24.5
	N2	19	18.6
	N3	9	8.8
	Not submitted	12	11.8
Hormonal status (ER/PR)	Positive/Positive	37	36.3
	Positive/Negative	8	7.8
	Negative/Positive	13	12.7
	Negative/Negative	44	43.2
HER2/neu status	Negative	67	65.7
	Positive	35	34.3

Table 2: Comprehensive Comparison of CD10 Expression with Clinicopathological and Molecular Parameters

Parameter / Subgroup	Category	CD10 Expression			Total
		Strong Positive	Weak Positive	Negative	
Age Group	<40	2	4	7	13
	41–60	16	9	10	35
	61–80	4	1	7	12
Tumor Size	<2 cm	0	0	0	0
	2–5 cm	15	9	11	35
	>5 cm	8	4	13	25
Histological Grade	Grade I	0	0	10	10
	Grade II	8	6	11	25
	Grade III	17	5	3	25
Histological Type	Invasive Breast Carcinoma NST	28	—	—	38
	Mucinous Carcinoma	0	—	—	9
	NST with Medullary Features	6	—	—	9
	Metaplastic Carcinoma	2	—	—	2
	Cribriform Carcinoma	0	—	—	1
	Adenoid Cystic Carcinoma	0	—	—	1
Lymphovascular Invasion (LVI)	Positive	18	9	15	42
	Negative	4	5	9	18
Lymph Node Status	Positive	17	10	14	41
	Negative	2	4	9	15
ER Status	Positive	0	1	15	16
	Negative	24	11	9	44
PR Status	Positive	1	2	11	14
	Negative	19	14	13	46
HER2/neu Status	Positive	17	8	6	31
	Negative	7	4	18	29
Triple Negative Status	Positive	7	3	5	15
	Negative	—	—	—	—

Table 3: Association of CD10 Expression(Chi-square test used) with Clinicopathological Parameters

Parameter	χ^2 Value	df	p-value	Interpretation
Age Group vs CD10	6.75	4	0.15	Not significant
Tumor Size vs CD10	2.63	2	>0.25	Not significant
Histological Grade vs CD10	25.20	4	<0.0001	Highly significant
Lymphovascular Invasion vs CD10	2.32	2	>0.30	Not significant
Lymph Node Status vs CD10	4.37	2	0.11	Not significant (trend)
ER Status vs CD10	26.55	2	<0.0001	Highly significant
PR Status vs CD10	11.60	2	0.003	Significant
HER2/neu vs CD10	11.45	2	0.003	Significant
Triple Negative Status vs CD10	1.78	2	>0.40	Not significant

A total of 302 breast specimens were evaluated, of which 102 cases (33.77%) were diagnosed as breast carcinoma. The majority of patients were aged 41–50 years (35.29%). Tumour size of 2–5 cm was most common (69.7%). Grade II tumours predominated (42.15%), with invasive carcinoma NST being the commonest subtype (75.49%). Lymphovascular invasion was present in 55.89% cases. ER/PR negativity and HER2 positivity were observed in 43.2% and 34.3% cases, respectively [Table 1]. The comparison of stromal CD10 expression with clinicopathological parameters showed a significant association with tumor aggressiveness. Strong CD10

positivity was predominantly observed in Grade III tumors, larger tumor size, invasive NST subtype, lymphovascular invasion, lymph node metastasis, HER2/neu positivity, and triple-negative breast carcinoma. In contrast, ER- and PR-positive tumors were largely CD10 negative, indicating that increased CD10 expression is associated with poor prognostic features [Table 2]. CD10 expression showed a significant association with histological grade ($p < 0.0001$), with higher-grade tumors exhibiting increased positivity. Significant associations were also observed with ER-negative ($p < 0.0001$), PR-negative ($p = 0.003$), and HER2-positive status ($p =$

0.003). No significant associations were found with age, tumor size, lymphovascular invasion, lymph node status, or triple-negative status, highlighting CD10 as a marker of aggressive tumor biology [Table 3].

DISCUSSION

The present study evaluated stromal CD10 expression in breast carcinoma and its association with clinicopathological and molecular prognostic parameters. The findings demonstrated that stromal CD10 expression was significantly associated with higher histological grade, estrogen receptor (ER) negativity, progesterone receptor (PR) negativity, and HER2/neu positivity, indicating that CD10 is a useful marker of aggressive tumor biology. These observations further support the concept that the tumor microenvironment, particularly activated stromal fibroblasts, plays a pivotal role in breast cancer progression, invasion, and metastasis through extracellular matrix remodeling and tumor–stromal interactions.^[11-13] In the present study, strong stromal CD10 expression was predominantly observed in Grade III tumors, whereas Grade I tumors were largely CD10 negative. Histological grade showed a highly significant association with CD10 expression ($p < 0.0001$). Similar findings were reported by earlier studies,^[11] who demonstrated that stromal CD10 expression was significantly associated with higher histological grade and poor disease-specific survival. Likewise, Kim et al,^[12] observed that stromal CD10 positivity correlated with increasing tumor grade, advanced stage, nodal metastasis, and reduced overall survival. Puri et al,^[13] also reported that stromal CD10 expression increased significantly with tumor grade and was strongly associated with Ki-67 positivity, suggesting increased proliferative activity. More recently, Dhande et al,^[14] demonstrated that CD10 positivity progressively increased from low-grade to high-grade breast carcinomas, confirming its role as an independent adverse prognostic marker. These observations indicate that increasing stromal CD10 expression reflects progressive stromal activation during tumor evolution. A highly significant inverse association between stromal CD10 expression and hormone receptor status was observed in the present study. ER-negative tumors exhibited significantly higher CD10 positivity than ER-positive tumors ($p < 0.0001$), while PR-negative tumors also demonstrated significantly increased stromal CD10 expression ($p = 0.003$). Similar observations have been consistently reported in previous studies. Makretsov et al,^[11] demonstrated that stromal CD10 expression was significantly associated with ER-negative tumors and poor clinical outcome. Puri et al,^[13] reported significant negative correlations between stromal CD10 expression and both ER and PR status. Kim et al,^[12] also observed that hormone receptor-negative tumors showed stronger stromal CD10 expression than receptor-

positive tumors. These findings suggest that stromal CD10 expression is associated with biologically aggressive breast cancers characterized by loss of hormone receptor expression and poor differentiation. HER2/neu-positive tumors in the present study exhibited significantly higher stromal CD10 expression ($p = 0.003$). Similar findings have been reported by Puri et al,^[13] who demonstrated a significant positive association between stromal CD10 expression and HER2 overexpression. Dhande et al,^[14] likewise observed significantly increased CD10 positivity in HER2-positive tumors and suggested that stromal CD10 should be included in routine histopathology reporting because of its prognostic significance. Kim et al,^[12] further demonstrated that stromal CD10 expression correlated with advanced tumor stage and HER2-driven aggressive biological behavior. These findings support the hypothesis that stromal CD10 promotes tumor invasion through degradation of extracellular matrix components and enhancement of tumor–stromal interactions. Although lymphovascular invasion and lymph node-positive tumors demonstrated relatively higher stromal CD10 expression in the present study, these associations did not achieve statistical significance. Makretsov et al,^[11] similarly reported no significant association between stromal CD10 expression and lymph node status. In contrast, Kim et al,^[12] demonstrated significant correlations between stromal CD10 expression, nodal metastasis, distant metastasis, and reduced survival. Dhande et al,^[14] also reported a significant association between stromal CD10 positivity and lymph node metastasis. These differences may be explained by variations in sample size, tumor stage, case selection, and immunohistochemical scoring methods across different studies. Nevertheless, the observed trend toward increased CD10 expression in node-positive and lymphovascular invasion-positive tumors in the present study supports its association with aggressive tumor behavior. Among the histological subtypes, invasive carcinoma of no special type (NST) demonstrated the highest stromal CD10 expression, whereas mucinous, cribriform, and adenoid cystic carcinomas showed absent or minimal expression. These findings are consistent with the recognized biological behavior of special histological subtypes, which generally exhibit lower stromal activation and better clinical outcomes than invasive NST. Similar observations have been described in previous immunohistochemical studies evaluating stromal biomarkers in breast carcinoma.^[13-16] Several recent studies have further emphasized the prognostic significance of stromal CD10 in breast carcinoma. Mohammadizadeh et al,^[15] demonstrated that stromal CD10 expression independently predicted poor prognosis and suggested its incorporation into routine pathological evaluation. Contemporary studies have also reported that persistent stromal CD10 expression following neoadjuvant chemotherapy is associated with poor

treatment response, indicating its potential predictive value.^[16-20] Collectively, these findings suggest that CD10 is not only a marker of tumor aggressiveness but may also represent a potential therapeutic target aimed at modifying the tumor microenvironment. Overall, the findings of the present study are in close agreement with previous literature and confirm that stromal CD10 expression is significantly associated with adverse clinicopathological and molecular prognostic factors in breast carcinoma. Its significant association with higher histological grade, ER negativity, PR negativity, and HER2 positivity highlights its potential role as a reliable prognostic biomarker. Routine assessment of stromal CD10 by immunohistochemistry may improve prognostic stratification and help identify patients with biologically aggressive tumors who may benefit from closer follow-up and targeted therapeutic strategies.

CONCLUSION

Stromal CD10 expression was significantly associated with higher histological grade, ER negativity, PR negativity, and HER2/neu positivity, indicating its association with aggressive breast carcinoma and poor prognosis. Stromal CD10 may serve as a simple, cost-effective prognostic biomarker and could be incorporated into routine histopathological evaluation for improved prognostic assessment.

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