

**CD10 Expression as a Prognostic Factor in Female Patients with Invasive Ductal Carcinoma of the Breast**Emad Sadaka<sup>1</sup>; Walid Almorsy<sup>1</sup> and Ayman Elsaka<sup>2</sup>Clinical Oncology Department<sup>1</sup>, Pathology Department<sup>2</sup> Faculty of Medicine, Tanta University, Gharbia, Egypt.  
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**Abstract Background:** CD10 is a cell surface metalloproteinase. CD10 expression in the stroma of various carcinomas was found to be related to aggressive behavior of these carcinomas. The aim of this study was to evaluate the prognostic impact of CD10 expression in female patients with invasive ductal carcinoma of the breast. **Patients and Methods:** This study included ninety seven patients with breast invasive ductal carcinoma. CD10 expression was detected by immunohistochemistry and scored based on the staining intensity and percentage of the stained cells. **Results:** CD 10 expression was significantly correlated with N stage ( $p<0.001$ ), ER status ( $p<0.001$ ), PR status ( $p<0.001$ ) tumor grade ( $p<0.001$ ), lymphovascular invasion ( $p<0.001$ ) and HER-2 expression ( $p<0.001$ ). While, there was no significant correlation with tumor size ( $p=0.113$ ), age ( $p=0.99$ ) and menstrual status ( $p=0.99$ ). The 5-years disease free survival (DFS) was 88.7% for negative CD10 expression and 20% for positive expression ( $p<0.001$ ). Multivariate analysis revealed that there was significant 5-years OS rate with CD 10 expression ( $p=0.003$ ). Meanwhile, there were significant 5-years DFS rate with CD 10 expression ( $p<0.001$ ), tumor size ( $p=0.01$ ) and lymphovascular invasion ( $p=0.006$ ). **Conclusion:** CD10 expression in invasive breast cancer patients was significantly correlated with N stage, ER status, PR status, tumor grade, lymphovascular invasion and HER-2 expression. CD 10 expression, tumor size and lymphovascular invasion were independent prognostic factors for invasive breast carcinoma. Thus, CD10 can be used as independent indicator for poor prognosis and can be used as a target for the development of novel therapies.

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**Key words:** invasive ductal carcinoma, CD10, prognosis

**1. Introduction**

Breast cancer is the most common cancer among women worldwide, and accounts for 18% of all female malignancies. Infiltrating ductal carcinoma is the most frequent histological type of breast cancer, accounting for approximately 68% of cases. <sup>(1)</sup>In Egypt, breast cancer constitutes 33% of all female cancers in Egyptian National Cancer institute (NCI). <sup>(2)</sup>

CD10 is a cell surface neutral endopeptidase that is not consistently expressed in the stromal cells of the normal breast. Although CD10 has been very useful in classifying the acute leukemias and subclassifying malignant lymphomas, frequent expression has been found in renal cell carcinoma, hepatocellular carcinoma, and carcinoma of the urinary bladder, prostate, the breast, stomach, and colon carcinoma. <sup>(3)</sup>

CD10 known as common acute lymphoblastic leukemia antigen (CALLA) is a 90 to 110 kDa cell surface zinc dependent metalloprotease that inactivates various kinds of biologically active peptides. CD10 positivity was reported in stromal myoepithelial cells from normal breast tissue and benign myoepithelial tumors. <sup>(4,5)</sup>

Several studies suggested that CD10 expression in tumor stroma is associated with biological

aggressiveness of the tumor. Expression of CD 10 in the stroma of invasive breast carcinoma is associated with ER-negativity, higher tumor grade, decreased patient survival, most significant in the node-negative subset. So, CD10 constitutes a clinically important prognostic marker and a potential target for development of novel therapies. <sup>(6-9)</sup>

Makretsov *et al.* (2007) examined a gene expression profiling of breast carcinoma stroma and identified two clinically significant types of stromal signatures in breast cancer and they found that CD10 expression was associated preferentially with desmoid-type fibromatosis stromal signature, and, possibly, contributed to a number of negative outcomes in invasive carcinoma of the breast with this type of stromal signatures. This may suggest that CD10 constitutes a component of a novel independent stromal signaling pathway, which contributes to biological and clinical aggressiveness of invasive breast carcinoma. <sup>(5)</sup>

**2. Patients and Methods**

This retrospective study was carried out at Clinical Oncology Department, Tanta University Hospital, between Jun 2007 and Jun 2010 on Ninety seven (97) female breast cancer patients with

histopathologically confirmed invasive ductal carcinoma.

Patients data were recorded including; age, menopausal status, pathology, tumor grade (G), tumor size (T), number of excised and invaded axillary lymph nodes (N), lymphovascular invasion (LVI), Estrogen receptors (ER), progesterone receptors (PR) and Her-2neu expression status. Blood chemistry(liver and renal functions tests), complete blood profile. Imaging studies (Chest X-ray, abdominopelvic ultrasound, CT, MRI and bone scanning were recorded.

#### **CD10 expression**

Blocks of formalin-fixed, paraffin-embedded tissue from these patients were retrieved from the Pathology department Tanta University Hospital. CD 10 antibody; mouse monoclonal antibody (Clone; 56C6) was used for immunostaining. A biotinylated secondary anti-immunoglobulin capable of binding to both the primary antibody and the streptavidin biotinylated system complex.

The chromogen used was 3,3-diaminobenzidine (DAB). One DAB tablet was dissolved in 10ml phosphate buffered saline. In a separate tube 0.2 ml hydrogen peroxide were added to 5.8 ml distilled water and was shaken well. Then 0.2ml diluted hydrogen peroxide solution was added to DAB solution and was mixed well. The buffer used was phosphate buffered saline (PBS) (pH 7.2).The counter stain is Mayer's and hematoxylin. Mounting media is Canada balsam.

Paraffin sections were put in xylene overnight and then heated in an oven at 52 °C for 15 minutes for deparaffinization. Sections were brought to distilled water through 2 changes of (100%), (95%) ethanol, and 10 minutes each for rehydration. Sections were incubated for 10 minutes with (3%) hydrogen peroxide in a humid chamber at room temperature. The slides were washed with PBS for 5 minutes.

Sections were placed in plastic Coplin jars containing citrate buffer solution, pH 6.0, and heated in the microwave oven for 10 minutes (two 5 minute cycles with interval of 1 minute between cycles to check the fluid level in the jars). If necessary more citrate buffer solutions is added after the first minutes to avoid drying out the tissue sections.

The Coplin jars are removed from the oven and allowed to cool for 15-20 minutes at room temperature. The slides were placed in PBS for 5 minutes. Two drops of primary antibody were put on each section. Slides were kept horizontal and incubated overnight in a humid chamber at room temperature. Excess reagent was thrown off and the slides were rinsed with 2 changes of PBS, 5 minutes each.

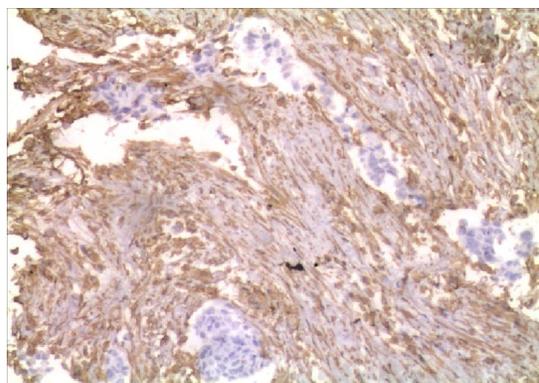
Two drops of biotinylated secondary antibodies were applied to each section for 30 minutes and incubated horizontally in a humid chamber at room temperature. The slides were washed with 2 changes of PBS, 5 minutes each. Two drops of preformed streptavidin biotinylated horseradish peroxidase complex were applied to each section for 30 minutes at room temperature. The slides were washed with 2 changes of PBS, 5 minutes each.

The antigen was finally localized by the addition of an appropriate precipitating chromogenic substrate. Two drops of DAB solution were applied to each section for 2-4 minutes at room temperature until the positive control showed brown precipitate. The slides were washed with distilled water for 5 minutes. Counterstaining was done using Mayer's hematoxylin for 30-60 seconds according to the intensity of blue coloration of the cell nuclei. The slides were washed with tap water.

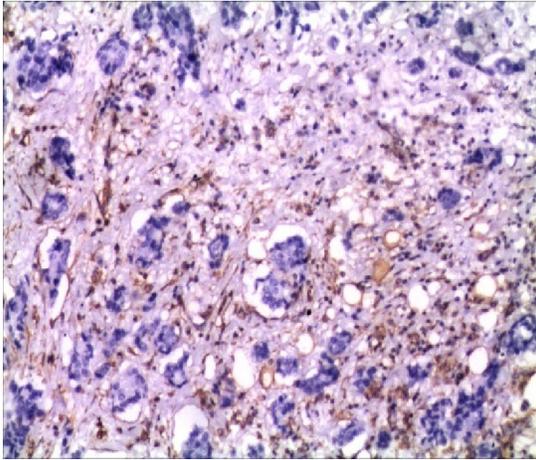
Sections were placed in 2 changes of (100%), (95%) ethanol, 10 minutes each. The slides were cleared in 2 changes of xylene, 10 minutes each. The slides were dried & cover slips were fixed using Canada balsam. In each staining session, previously known sections to be positive for CD10 from breast carcinoma were used as a positive control. As a negative control, sections were processed through the above sequence with omission of primary antibody and instead PBS was added.

#### **Evaluation of CD10 Immunostaining**

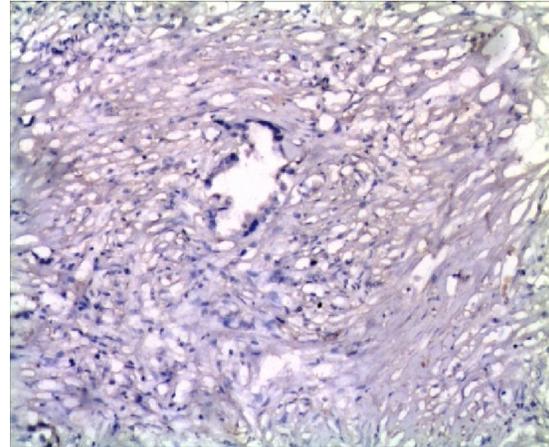
Every section was carefully examined at power magnification (x200) for the presence of tumor stromal immunostaining. The CD10 immunostaining was scored quantitatively as positive CD10 for specimens with more than 10% of the tumor stromal cells, strong positive CD10 when there is strong staining more than 30% of tumor stromal cells, figure (1a) and weak positive CD10 if staining was between 10% to 30%, figure (1b).<sup>(7)</sup>



**Figure (1a):** strong cytoplasmic stromal CD10 expression (Streptavidin biotin X 200 )



**Figure (1b):** weak cytoplasmic stromal CD10 expression (Streptavidin biotin X 200 )



**Figure (1c):** negative stromal CD10 expression (Streptavidin biotin X 200 )

### Statistical analysis

All data were statistically analyzed using the Statistical Package for the Social Sciences, version 21.0 (SPSS Inc., Chicago, IL, USA). The correlation between clinicopathological features and CD10 expression was compared using a Chi-square test.

The Cox proportional-hazards model was used for univariate and multivariate analyses to identify the independent prognostic factors for Overall survival. Overall survival (OS) was calculated by the Kaplan-Meier method, and the differences in survival rates were analyzed by the log-rank test. Statistically, *P* value less than 0.05 was considered to be significant.

### 3. Results

This study evaluated a total of 97 female patients with invasive ductal carcinoma of the breast. Patients' age ranged from 24 years to 75 years (median 50 years, mean  $50.5 \pm 11.43$  years) with follow up period ranged from 5 to 8 years.

Table (1) showed the correlation between the CD 10 expression and clinicopathologic characteristics; CD 10 expression was significantly correlated with N stage ( $p < 0.001$ ), ER status ( $p < 0.001$ ), PR status ( $p < 0.001$ ) tumor grade ( $p < 0.001$ ), lymphovascular invasion ( $p < 0.001$ ) and HER-2 expression ( $p < 0.001$ ). While, there was no significant correlation with tumor size ( $p = 0.113$ ), age ( $p = 0.99$ ) and menstrual status ( $p = 0.99$ ).

Figures (2&3) Showed that the 5-years overall survival (OS) and Disease free survival (DFS) in all patients were 71% and 62.9% respectively. Figure (3) Showed that the 5-years OS among all patients

according to CD10 expression was 95.2% for negative expression and 28.6% for positive expression ( $p < 0.001$ ). Figure (4) showed that the 5-years DFS among all patients according to CD10 expression was 88.7% for negative expression and 20% for positive expression ( $p < 0.001$ ).

As shown in table (2) in univariate analysis, there was significant impact on 5-year OS rate with CD10 ( $p < 0.001$ ), T stage ( $p = 0.011$ ), N stage ( $p < 0.001$ ), ER ( $p < 0.001$ ), PR ( $p < 0.001$ ), Her-2/neu ( $p < 0.001$ ), grade of differentiation ( $p < 0.001$ ) and lymphovascular invasion ( $p = 0.012$ ). Age and menstrual status showed insignificant correlation with 5 year OS rate.

The univariate analysis in table (3) showed a significant impact on 5-year DFS rate with CD10 ( $p < 0.001$ ), Tumor size ( $p = 0.02$ ), N stage ( $p < 0.001$ ), ER ( $p < 0.001$ ), PR ( $p < 0.001$ ), Her-2/neu ( $p < 0.001$ ), grade of differentiation ( $p < 0.001$ ) and lymphovascular invasion ( $p = 0.021$ ). Age and menstrual status showed insignificant correlation with 5 year DFS rate ( $p = 0.219$  &  $0.169$ ) respectively.

A multivariate analysis using the Cox proportional hazard regression model was performed. As shown in Table (3), there was significant 5-years OS rate with CD 10 expression ( $p = 0.003$ ). Meanwhile, there were significant 5-years DFS rate with CD 10 expression ( $p < 0.001$ ), tumor size ( $p = 0.01$ ) and lymphovascular invasion ( $p = 0.006$ ).

Median overall survival was 68 (range, 16-96) months with 71.1% 5-year OS. Median disease free survival was 67 (range, 11-96) months with 63.9% 5-year DFS.

**Table (1): Patient characteristics according to CD10 expression**

Characters	CD -ve 62 (63.9%)	CD +ve 35 (36.1%)	<i>p</i>	All group 97 (100%)
<b>Age</b>				
≤50 years	33 (53.2%)	18 (51.4%)	0.99	51 (52.6%)
>50 years	29 (46.8%)	17 (48.6%)		46 (47.4%)
<b>Menstrual status</b>				
Menstruating	32 (51.6%)	18 (51.4%)	0.99	50 (51.5%)
Menopause	30 (48.4%)	17 (48.6%)		47 (48.5%)
<b>Estrogen receptor</b>				
+ve	58 (93.5%)	14 (40%)	<0.001	72 (74.2%)
-ve	4 (6.5%)	21 (60%)		25 (25.8%)
<b>Progesterone receptor</b>				
+ve	56 (90.3%)	13 (37.1%)	<0.001	69 (71.1%)
-ve	6 (9.7%)	22 (62.9%)		28 (28.9%)
<b>Her-2/neu</b>				
+ve	8 (12.9%)	19 (54.3%)	<0.001	27 (27.8%)
-ve	54 (87.1%)	16 (45.7%)		70 (72.2%)
<b>T stage</b>				
≤ 5	46 (74.2%)	20 (57.1%)	0.113	66 (68%)
> 5	16 (25.8%)	15 (42.9%)		31 (32%)
<b>N stage</b>				
0	20 (32.3%)	1 (2.9%)	<0.001	21 (21.6%)
1	22 (35.5%)	6 (17.1%)		28 (28.9%)
2	18 (29%)	18 (51.4%)		36 (37.1%)
3	2 (3.2%)	10 (28.6%)		12 (12.4%)
<b>Grade</b>				
1-2	58 (93.5%)	15 (42.9%)	<0.001	73 (75.3%)
3	4 (6.5%)	20 (57.1%)		24 (24.7%)
<b>Lymphovascular invasion</b>				
+ve	19 (30.6%)	24 (68.6%)	0.001	43 (44.3%)
-ve	43 (69.4%)	11 (31.4%)		54 (55.7%)

**Table (2) Univariate & multivariate analysis of factors affecting Overall Survival rate**

Factor		5-year OS	HR (95% CI)	<i>p</i>
<b>Univariate Analysis</b>				
Age	≤50 years	66.7%	-	0.256
	>50 years	76.1%		
Menstrual Status	Menstruating	66%	-	0.207
	Menopause	76.6%		
ER	+ve	91.7%	21.787 (9.159-51.821)	<0.001*
	-ve	12%		
PR	+ve	91.3%	15.778 (6.690-37.211)	<0.001*
	-ve	21.4%		
Her-2/neu	+ve	40.7%	0.227 (0.109-0.474)	<0.001*
	-ve	82.9%		
T stage	≤ 5	78.8%	2.521 (1.231-5.164)	0.011*
	> 5	54.8%		
N stage	0	95.2%	2.345 (1.535-3.580)	<0.001*
	1	82.1%		
	2	58.3%		
	3	41.7%		
Grade	1-2	84.9%	2.703 (1.865-3.915)	<0.001*
	3	29.2%		
LVI	+ve	58.1%	0.387 (0.184-0.814)	0.012*
	-ve	81.5%		
CD10	Low	95.2%	19.672 (6.730-57.499)	<0.001*
	High	28.6%		
<b>Multivariate Analysis</b>				
CD10			8.631 (2.086-35.717)	0.003*

ER: Estrogen receptor; PR: Progesterone receptor; LVI: Lymphovascular invasion; HR (95%CI): Hazard Ratio (95% Confidence Interval); \*Significant <0.05

**Table (3) Univariate & multivariate analysis of factors affecting disease free survival rate**

Factor		5-year DFS	HR (95% CI)	p
<b>Univariate Analysis</b>				
Age	≤50 years	58.8%	-	0.219
	>50 years	69.6%		
Menstrual Status	Menstruating	58%	-	0.169
	Menopause	70.2%		
ER	+ve	84.7%	20.536 (9.517-44.268)	<0.001*
	-ve	4%		
PR	+ve	84.1%	12.654 (6.071-26.373)	<0.001*
	-ve	14.3%		
Her-2/neu	+ve	22.2%	0.204 (0.103-0.406)	<0.001*
	-ve	80%		
T stage	≤ 5	71.2%	2.211 (1.135-4.307)	0.020*
	> 5	48.4%		
N stage	0	95.2%	2.063 (1.413-3.012)	<0.001*
	1	64.3%		
	2	55.6%		
	3	33.3%		
Grade	1-2	79.5%	2.687 (1.907-3.785)	<0.001*
	3	16.7%		
LVI	+ve	51.2%	0.450 (0.228-0.886)	0.021*
	-ve	74.1%		
CD10	Low	88.7%	13.434 (5.763-31.317)	<0.001*
	High	20%		
<b>Multivariate Analysis</b>				
T stage			2.783 (1.276-6.074)	0.010*
LVI			4.123 (1.512-11.239)	0.006*
CD10			9.342 (2.799-31.181)	<0.001*

ER: Estrogen receptor; PR: Progesterone receptor; LVI: Lymphovascular invasion; HR (95%CI): Hazard Ratio (95% Confidence Interval)\*Significant <0.05

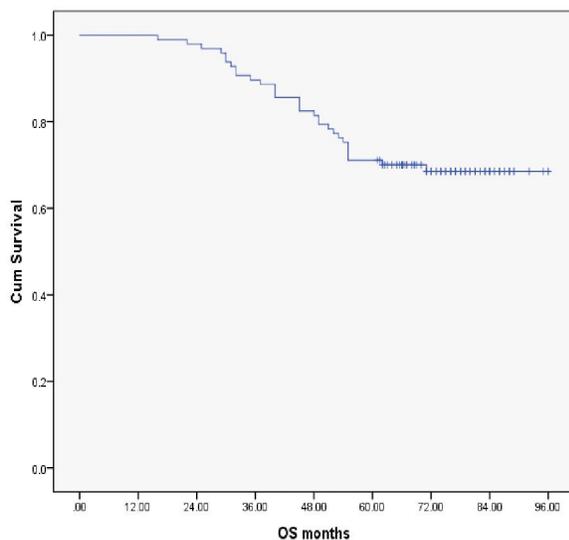


Fig (2): Overall survival for all patients

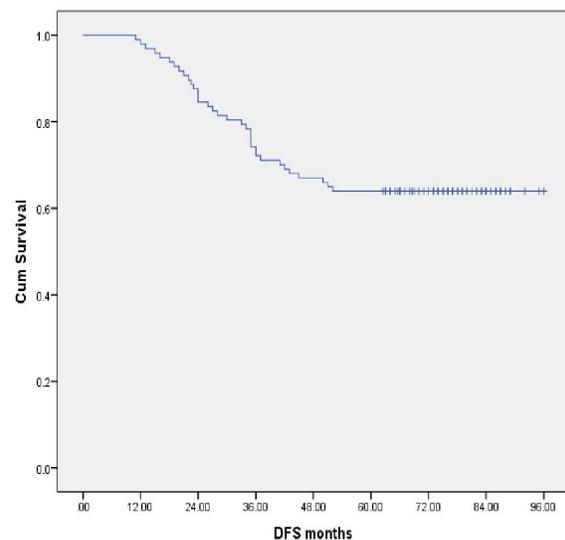


Fig (3): Disease free survival for all patients

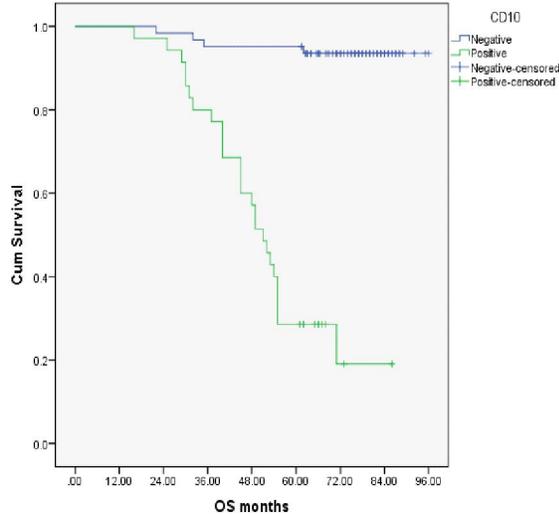


Fig (4): Overall survival for all patients according to CD10 expression

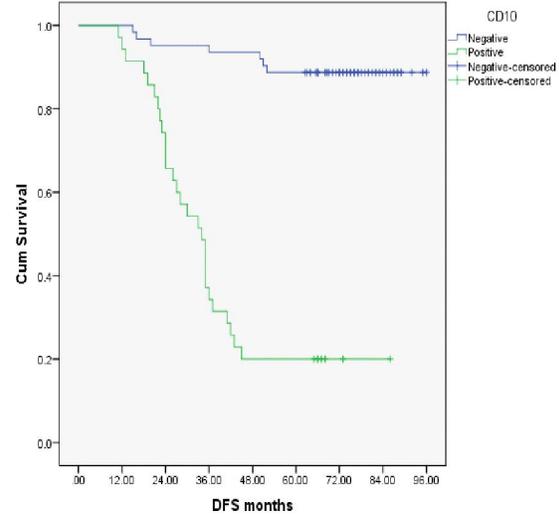


Fig (5): Disease free survival for all patients according to CD10 expression

#### 4. Discussion

In this study, among 97 female patients with invasive ductal carcinoma of the breast, CD 10 expression was significantly correlated with N stage ( $p < 0.001$ ), ER status ( $p < 0.001$ ), PR status ( $p < 0.001$ ) tumor grade ( $p < 0.001$ ), lymphovascular invasion ( $p < 0.001$ ) and HER-2 expression ( $p < 0.001$ ). While, there was no significant correlation with tumor size ( $p = 0.113$ ), age ( $p = 0.99$ ) and menstrual status ( $p = 0.99$ ).

The association between the expression of CD10 and overall survival. The 5 years overall survival in patients with negative CD10 expression was 95.2%, which was significant different from that observed in patients with positive CD10 expression which was 28.6% ( $p < 0.001$ ). The multivariate analysis according to 5-years DFS revealed that CD 10 expression ( $p < 0.001$ ), tumor size ( $p = 0.01$ ) and lymphovascular invasion ( $p = 0.006$ ) were independent prognostic factors for invasive breast carcinoma.

Mohammadizadeh *et al.* (2012) evaluated forty nine patients with invasive breast carcinoma and no association was found between stromal CD10 expression and age, carcinoma subtype, and HER2/neu status. A significant positive correlation was seen between stromal CD10 expression and tumor size ( $p = 0.01$ ), axillary lymph node status ( $p = 0.02$ ), and tumor grade ( $p = 0.004$ ). Although negative correlations were detected between stromal CD10 expression and estrogen receptor and progesterone receptor status, these correlations were not statistically significant. They suggest a strong effect of stromal CD10 expression on aggressive behavior of breast carcinoma and introduce this marker as a potential prognostic determinant in breast cancer.<sup>(4)</sup>

Taghizadeh *et al.* (2014) evaluated One hundred patients with histopathologic diagnosis of invasive ductal carcinoma and they found that, stromal CD10 expression in IDC has significantly correlated with increasing tumor size ( $p < 0.001$ ), increasing histologic grade ( $p < 0.001$ ), the presence of nodal metastases ( $p < 0.001$ ) and estrogen receptor negative status ( $p = 0.003$ ). They concluded that, stromal CD10 expression in IDC has closely correlated with invasion and metastasis and it might play an important role in the pathogenesis of IDC.<sup>(10)</sup>

Puri *et al.* (2011) evaluated CD10 expression in fifty patients with invasive breast carcinoma and they found that CD10 was found to be positive in stroma of 40/50 (80%) cases. Stromal CD10 showed positive correlation with tumour grade however it was not statistically significant ( $p = 0.1390$ , with HER2-neu ( $p = 0.001$ ), and with ki67 ( $p = 0.027$ ) and negative correlation with ER and PR. They concluded that hence CD10 expression correlated strongly with HER2-neu and ki67 positivity, ER/PR negativity, and higher tumour grade, thus indicating that CD10 can be used as independent marker indicating poor prognosis and can be used as target for the development of novel therapies.<sup>(11)</sup>

Iwaya *et al.* (2002) evaluated 123 patients with breast cancer and The frequency of positive stromal staining was significantly higher in the cases with axillary lymph-node metastasis ( $p = 0.038$ ), but there were no correlations between stromal CD10 expression and age, tumor size, histologic grade, or clinical stage. The patients whose tumors contained CD10-positive stromal cells had a shorter metastasis-free interval ( $p = 0.0008$ ). In the multivariate analysis, only CD10 remained a significant predictor for time to

recurrence ( $p=0.0059$ ), and CD10 was the single significant prognostic factor for overall survival in the univariate analysis ( $p=0.0021$ ).<sup>(3)</sup>

Makretsov *et al.* (2007) evaluated 438 cases of invasive breast carcinoma. There were correlations between stromal CD10 expression and higher tumor grade ( $p=0.01$ ) and estrogen receptor (ER) negative status ( $p=0.002$ ). There was no correlation between CD10 and lymph node status, tumor size, histological subtype, progesterone receptors, and Her2 status. Stromal CD 10 expression was associated with decreased long-term disease-specific and overall survival in the entire cohort ( $p=0.01$ ), and in lymph node negative ( $p=0.05$ ), but not lymph node positive subset of patients. It approached prognostic significance in multivariate analysis ( $p=0.06$ ). Thus, stromal CD10 expression in invasive carcinoma of the breast is associated with ER negativity, higher tumor grade and decreased survival and constitutes a potential prognostic marker and a target for development of novel therapies.<sup>(5)</sup>

### Conclusion

CD10 expression in invasive breast cancer patients was significantly correlated with N stage, ER status, PR status, tumor grade, lymphovascular invasion and HER-2 expression. CD 10 expression, tumor size and lymphovascular invasion were independent prognostic factors for invasive breast carcinoma. Thus CD10 can be used as independent indicator for poor prognosis and can be used as target for the development of novel therapies. Further multicentric studies of bigger number of patients still needed.

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