The correlation of stromal CD 10 expression in invasive breast cancer with various clinico-pathological parameters

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Abstract

Background: CD10 is a cell surface zinc-dependent enodpepitdase and its expression in tumor stroma is thought to be associated with biological aggressiveness of many epithelial malignancies.

Objectives: To estimate the frequency of stromal CD10 expression in invasive breast cancer and its correlation to clinico-pathological data including immunohistochemical markers.

Methods: This study included 102 cases of invasive breast carcinoma. CD10 expression was assessed by immunohistochemistry and scored as negative, weak and strong positive and the results were statistically analyzed in correlation to various clinical and pathological parameters in addition to other immunohistochemical markers.

Results: CD 10 was found to be positive in 58/102 cases (56.8%). Stromal CD10 showed significant correlation with the T parameter of the TNM staging system (p=0.043), dermal infiltration (p=0.049) and estrogen receptor positivity (p=0.039), while there was no significant correlation with the patients' age (p=0.20), tumor grade (p=0.294), and lymph node status (p=0.29).

Conclusion: Stromal CD10 was expressed in more than half of cases with invasive breast carcinoma, and there was a significant correlation of the stromal CD 10 expression with the T parameter of the TNM staging system, dermal infiltration and the positivity of estrogen receptor expression and no correlation was found between stromal CD10 expression and patients' age, tumor grade, stage, lymph node status, lymphovascular invasion, perineural invasion, Paget's disease of the nipple, progesterone receptors expression and HER2/Neu status.

Keywords: Breast, CD10, Cance

Introduction

The prevailing view of breast tumor progression is tumor epithelial cell driven, because tumor epithelial cells acquired genetic alterations and instability of their genomic material, and the most aggressive invasive cells are driven to proliferation due to clonal selection. But recent studies demonstrate genetic alterations in tumor stroma, a finding that raises the possibility that clonal selection occurs in non- epithelial cells and the tumor microenvironment may play an active role in driving tumor progression in a "team effort"[(1).]

Myoepithelial cell layer is present in both benign lesions and in in situ carcinoma whereas its loss is the golden criterion that marks invasive cancer. Of the many markers of myoepithelial cells is the 90-100 kDA cell surface zinc-dependent peptidase or the CD 10. This peptidase is commonly expressed in stromal myoepithelial cells from normal breast tissue, in 30% of DCIS and is completely lost in invasive breast cancer [(2).]

From the very early studies, it was obvious that CD10 is not restricted to hematopoietic malignancies but is also expressed by some fetal tissues and solid tumors of organs [(3-8).] In breast cancer, the use of CD10 for diagnosis and prognosis is more complex. A study of 600 tumors samples from 200 patients with invasive breast cancer has demonstrated that CD10 overexpression correlates with improved disease-free survival and fewer metastases [(9)]. Some authors, conversely, think that in invasive breast carcinoma CD10 is also abnormally expressed by environmental stromal cells, which contributes to obscure and apparently controversial interpretation. CD10 expression by the stromal cells is then correlated with poor prognosis, estrogen receptor negativity, and high grade [(5).]

The aims of this study are to estimate the frequency of stromal CD10 expression in invasive breast cancer and to examine the correlation of stromal CD10 expression with variable clinico-pathological data including; age, histological type, grade, stage, lympho-vascular invasion, nipple invasion, dermal infiltration, lymph node status, ER, PR and HER2/Neu.

Methods

This is a clinic-pathological study carried out in the histopathology department at the Central public health laboratory in Duhok-Iraq over the period from 2010 to 2016. Any case with missing of important data was excluded from the study.

This study included 102 patients diagnosed with invasive breast carcinoma who underwent mastectomy with or without axillary clearance. Paraffin embedded, pretreatment tumor tissue was available for the 102 patients with invasive breast carcinoma. All patients were newly diagnosed and hadn't received any previous treatment.

Specimens were already fixed in 10% formalin, processed and embedded in paraffin wax, cut at five micron-thickness and stained by hematoxylin and eosin. Stained sections were reviewed for determining the histological type and tumor grade for

invasive ductal carcinoma, additional sections were taken for IHC (ER, PR&HER2/Neu).

One block, that showed predominant stromal reaction around the tumor cells, was selected per case [10-12] and stained for CD10. Labeled polymer and enhanced polymer systems (Dako EnVision™ Flex) method according to Dako recommendation was used to stain the tissue by CD10 antibody. The instruments used in IHC staining were Manual microtome, Dako PT link, Dako autostainer link 48 and Olympus CX22 light microscope.

The staining of the cell membrane and the cytoplasm of the stromal cells were semi quantitatively scored as:

1. Negative (no staining or less than 10% of stromal cell).
2. Weak positive (either diffuse weak staining or strong focal staining more than 10% and less than 30% of the stromal cells per slide).
3. Strong positive (defined as strong staining of 30% or more of the stromal cells per slide).
4. The slides were scored independently, Non-neoplastic myoepithelial and epithelial cells in normal parenchyma adjacent to the tumor, used as built-in positive and negative controls respectively [13].

The collected data was analyzed using SPSS version 22 (SPSS Inc., Chicago, USA). Each parameter was evaluated independently by measuring the mean and standard deviation. And these parameters were expressed in frequency and percentages. Association between stromal CD10 expression and clinico-pathological parameters (age, histological subtype, tumor grade, size, lymphovascular invasion, and lymph node status and tumor stage) were evaluated using the Anova test and independent T test with a confidence limit of 95% and a P value equal or less than 0.05 was considered significant.

Results

This study included 102 mastectomy specimens, the ages of the patients range from 20-80 with a mean of 47.72 year, and 36 cases were in the 5th decade (Figure 1).

Histologically, 100 cases (98.03%) were invasive ductal carcinoma and 2 cases (1.96%) were invasive lobular carcinoma.

Regarding the grade only 15 cases (14.7%) had grade I tumors, 41 patients (40.2%) grade 2 and 46 patients (45.1%) grade 3.

The greatest dimension of the tumors from 8-130 mm with a mean of 43.70 mm, and 17 patients (16.67%) had tumors equal to or less than 20 mm, 58 patients (56.86%) had tumors between 21-50 mm and the rest 27 patients (26.47) had tumors more than 50 mm.

According to the TNM staging system, 53 patients (51.9%) were included in the T2 category (Figure 2), while 34 cases (33.3%) had no nodal involvement (Figure 3).

More than half of the cases (52.9%) were in advanced stage (Stage 3) and (table 1) shows the stage of the patients.

Lymphovascular invasion was detected in 63 cases (61.7%). Dermal infiltration and perineural invasion were seen in 12 and 18 cases respectively, while Paget's disease of the nipple was seen in 7 cases. ER, PR and Her2/Neu positivity was seen in 71 cases (69.9%), 69 cases (67.6%) and 54 cases (52.9%) respectively.

Stromal CD10 expression was detected in (56.8%) of cases and strong expression was found in 39 cases (38.2%) (Figure 4), weak expression in 19 cases (18.6%) (Figure 5) and negative in 44 patients (43.2%) (Figure 6).

Despite the wide range difference in the patients' age, no significant association was found between the stromal CD10 expression and the age of the patients (p= 0.20), similarly with the tumor size (p=0.47) and presence of Paget's disease of the nipple (p=0.6).

There was a statistically significant correlation between the stromal CD10 expression and the skin ulceration (p=0.04) which was detected in (13) patients.

There is a significant statistical correlation between stromal CD10 expression and the T parameter of the TNM staging system (p= 0.043), while there was no significant correlation with the N parameter (p =0.29).

Although there was a strong expression of the stromal CD10 in the high grade tumors but no statistical significance was found between the stromal CD10 expression and the tumor grade (p=0.294).

No significant association was demonstrated between the stromal CD10 expression and the tumor stage (p = 0.184), lymphovascular invasion (p = 0.117) and perineural invasion (p=0.118).

Dermal infiltration was statistically correlated with the stromal CD10 expression (p= 0.049).

Stromal CD10 expression was found to be significantly correlated with positive ER expression (p= 0.039), while no statistically significant correlation was observed with PR expression (p = 0.313) and Her2/Neu (p =0.98).

Tables (2,3,4) summarize the statistical correlation of stromal CD10 and various clinicopathological parameters.

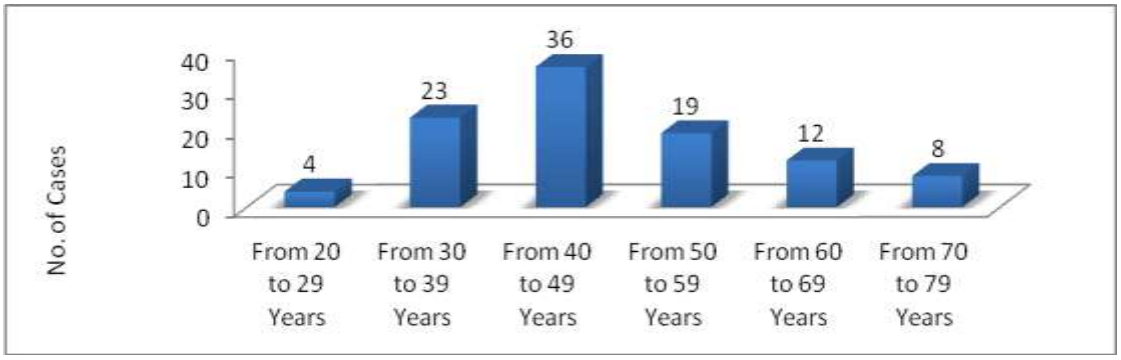

Figure 1: The age distribution of the included patients.

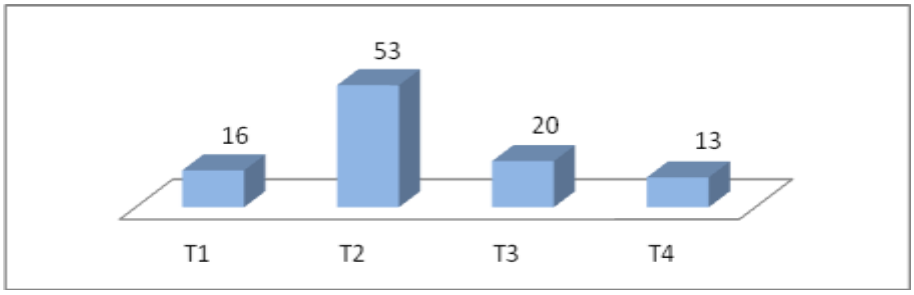

Figure 2: The T parameter (TNM) distribution of the included patients.

Table 1: The surgical stages of the included patients.

Stage I		No. of cases 10	% 9.8
II	IIa	21	20.5
	IIb	17	16.6
III	IIIa	24	23.5
	IIIb	10	9.8
	IIIc	20	19.6
Total		102	100

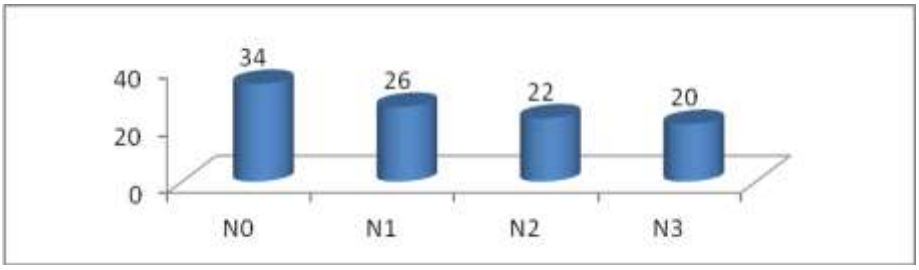

Figure 3: The nodal status (TNM) of the incorporated cases.

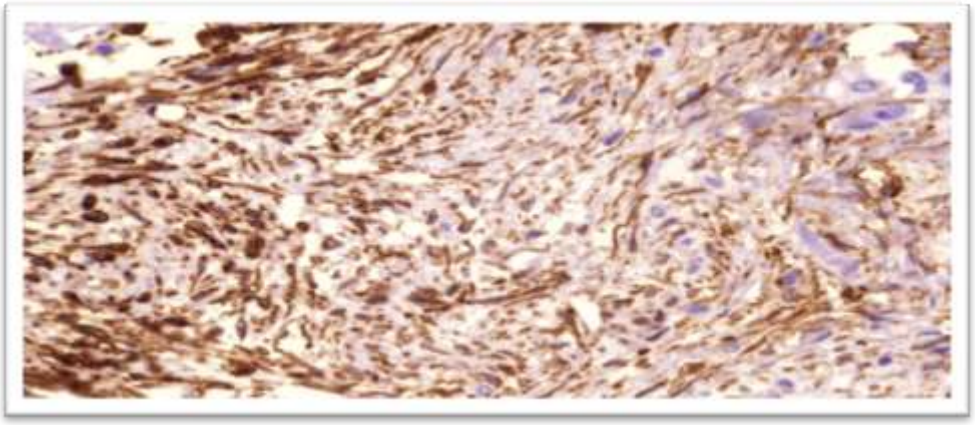

Figure 4: CD10 expression strong positivity (IHC x40).

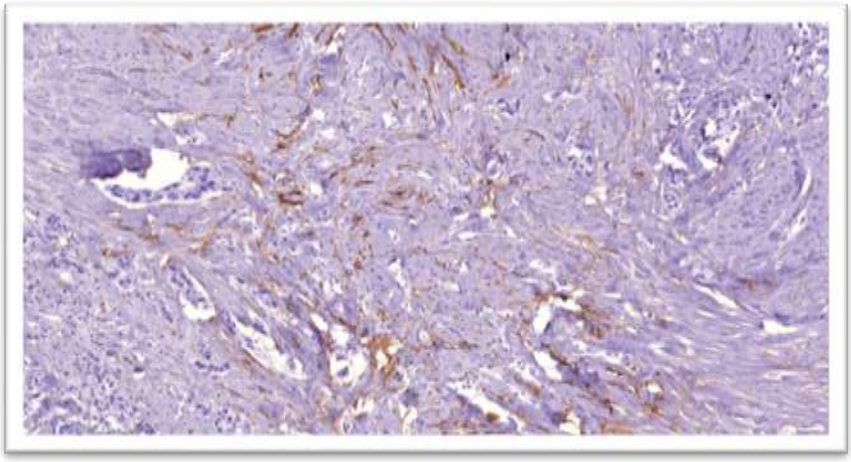

Figure 5: Invasive breast carcinoma with weak stromal CD10 expression (IHC x10).

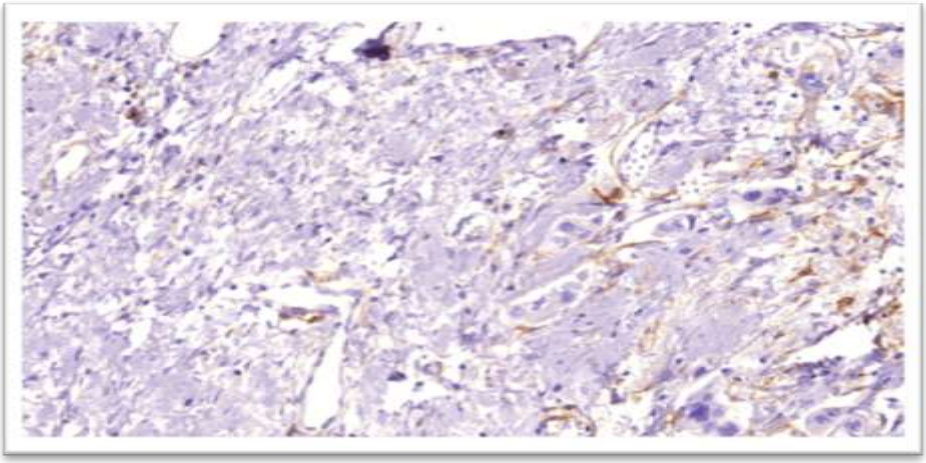

Figure 6: Negativity of CD10 immunostaining in the stroma among tumor cell clusters (IHCx20).

Table 2: Statistical correlation of stromal CD 10 expression and the clinical parameters

Clinical parameter	P value
Age	**0.2**
Tumor size	**0.47**
Paget's disease	**0.6**
Skin Ulceration	**0.04**

Table 3: Statistical correlation of stromal CD 10 expression and the pathological parameters

Pathological parameter	P value
T of TNM	**0.043**
N of TNM	**0.29**
Grade	**0.294**
Stage	**0.184**
Lymphovascular invasion	**0.117**
Perineural invasion	**0.118**
Dermal infiltration	**0.049**

Table 4: Statistical correlation of stromal CD 10 expression and the immune markers

Immune marker	P value
ER	**0.039**
PR	**0.313**
Her2/Neu	**0.98**

Discussion

Needless to describe the alarming data from all over the world about breast cancer incidence, prevalence and mortality [14-16], breast cancer remains a major concern for population, governments, patients and their families, surgeons, oncologists, other workers in the medical field and researchers. In this era there is increasing interest in biomarkers which may have diagnostic, therapeutic or prognostic implications. One of the newly emerging biomarker is the CD10 that is expressed by the stromal cells.

The results of this study showed that about two third of the cases (56.8%) were positive for stromal CD10, a figure which is a little bit lower than the 80% positivity reported by Puri *et al* [13], the 79% of Marketsov *et al* [5], 74% of Hosni *et al* [17], 81.6% of Mohammadizadeh *et al* [18], but comparable to the 64% of Taghizadeh-Kermani *et al* [19] , but greatly dissimilar to 28.6 % positivity reported by Sadake *et al* [20] . Some authors used different scoring system and accordingly their results were different. Masaki *et al* [21], Iwaya *et al* [22].

These differences in the results of stromal CD10 expression can be attributed to technical factors or to the accuracy of staining methods. We failed to demonstrate a statistical significant correlation between the stromal CD 10 expression and the age of the patient, as did Iwaya *et al* [22] Sadaka *et al* [20] and Hosni *et al* [17]. Researches gave different results regarding the relation between stromal CD10 expression and the tumor size, while some failed to demonstrate any correlation (Makretsov *et al* [5] Iwaya *et al* [22] Masaki *et al* [21] Puri *et al* [13] Sadaka *et al* [20] and Hosni *et al* [17] and this in agreement with our study. Others demonstrated some correlation

Kim *et al* [23] Mohammadizadeh *et al* [18] and Taghizadeh-Kermani *et al* [19].

As far as the grade is concerned we failed to demonstrate a significant correlation between stromal CD10 and the grading despite the fact that 46 cases in this study were grade III, and most of strong positive stromal CD10 were also grade III. Similar results have been demonstrated by other studies including Iwaya *et al* [22] and Puri *et al* [13]. Divergent results recorded by Makretsov *et al* [5] Kim *et al* [23] Mohammadizadeh *et al* [18] Taghizadeh-Kermani *et al* [19] Sadaka *et al* [20] and Hosni *et al* [17] showed a statistically significant positive correlation between stromal CD10 expression and tumor grade.

On the contrary of Sadaka *et al* [20] study, we were unable to report a statistically significant correlation between stromal CD10 expression and lymphovascular invasion. Most of the studies didn't include perineual invasion and dermal infiltration in their work; however in this study we found a statistical significant correlation between stromal CD10 expression and dermal infiltration while there was no statistical significant correlation between stromal CD 10 expression and the presence of perineural invasion. Our findings, concerning stromal CD10 expression with the T parameter in TNM staging system showed a statistically significant correlation, this result is in agreement with other studies including Kim *et al* [23] Mohammadizadeh *et al* [18] and Taghizadeh-Kermani *et al* [19], on the other hand Makretsov *et al* [5] Iwaya *et al* [22]Masaki *et al* [21] Puri *et al* (13)Sadaka *et al* [20] and Hosni *et al* [17] failed to show a significant correlation between stromal CD10 expression and T parameter of TNM staging system.

The correlation of Stromal CD10 expression and N parameter (lymph node status) in TNM staging system:

As far as the lymph node involvement, the results of this study agreed with Makretsov *et al* [5] and Hosni *et al* [17] who failed to establish a positive correlation between stromal CD10 expression and lymph node status. On the contrary studies done by Iwaya *et al* [22] Masaki *et al* [21] Mohammadizadeh *et al* [18] Taghizadeh- Kermani *et al* [19] Sadaka *et al* [20] and Kim *et al* [23] demonstrated a statistically significant correlation between stromal CD10 expression and the nodal status. The correlation between stromal CD10 expression and tumor stage:

In the same line, no association was demonstrated between stromal CD10 expression and the cancer stage, a result similar to the one found in the study done by Iwaya *et al* [22].The reason behind such non-significant results is probably that Iwaya *et al* did not include stage IV in their selected cases as same as our study.

An important finding in the current study is that stromal CD10 expression was found to show a statistical significant correlation with ER positive cases. This is different from Taghizadeh-Kermani *et al* [19] and Makretsov *et al* [5] who find a significant correlation with ER negativity. Moreover, no statistically significant correlation was found between stromal CD10 expression and PR positive cases a result similar to the results of Makretsov *et al* [5].

Similarly as we haven't been able to find a significant correlation between stromal CD10 expression and HER2/Neu status, Makretsov *et al* [5] documented the same results.

The dilemma of results variability could be attributed to plethora factors; the first and most important suggested factor is that CD10 is recently introduced to the field of breast cancer biology, and the second is the lack of standardization of evaluation unlike ER, PR and HER2/Neu. Other factors could include various technical issues and difference in the methodology.

The conclusion which can be made from the present work is that Stromal CD10 expression is correlated with the T parameter of the TNM staging system, with dermal infiltration and ER positivity while it is unrelated to the patients' age, tumor grade, tumor stage, lymph node status, lymphovascular invasion, perineural invasion, PR positivity and HER2/Neu expression. One could recommend a follow up study to determine the correlation of stromal CD10 expression and survival of the patients to determine whether it is independent prognostic factor or not.

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