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Prognostic Factors in Breast Carcinoma in Specific Reference to CD 10

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1. Introduction

Breast cancer is the most common cancer in urban population and stands next to cervical cancer among the rural population. More than one million cases occur worldwide annually and is the leading cause of death in women. Most women are diagnosed between 25 to 45 years of age[1]. Human breast cancers depend upon estrogen and/or progesterone for their growth and are mediated through estrogen and progesterone receptors.

Recent studies show genetic alterations in tumor stroma, with the clonal selection occurring in non-epithelial cells and the active role in driving tumor progression is laid by tumor microenvironment. There were evidences showing myofibroblasts play a great role in tumor growth, invasion and metastasis.

CD 10, one of the myoepithelial markers is a zinc-dependent peptidase expressed in myoepithelial cells of normal breast tissue, DCIS (30%) and is lost in invasive carcinoma breast. Abnormal CD 10 expression is correlated with poor prognosis, high grade and estrogen receptor negativity[2] and more biologically aggressiveness of tumors.

Since stromal cells are not as genetically unstable as cancer cells, the tumor microenvironment has been considered as a potential therapeutic target because they less likely to develop resistance.

2. Aims and Objectives

1) To estimate the frequency of stromal CD10 expression in Breast carcinoma.
2) To assess the prognostic significance of stromal CD10 expression.
3) To assess the correlation of CD10 expression with tumor size, lymphnode status, histological grade, ER, PR, HER2 neu status in Breast carcinoma.

3. Materials and Methods

The present Study design is a Prospective study done during the time period from June 2020 to May 2022 in Department of Pathology, Madurai Medical College, Madurai. Specimens were obtained from the patients who have undergone modified radical mastectomy and the core needle tru cut biopsies done preoperatively and confirmed as invasive carcinoma breast NST by histopathological examination. The institutional ethical committee approval was obtained.

Sample size

A total of 895 breast biopsies were received in our study period. Of which 380 cases were diagnosed as malignant lesions of the breast. Out of which 100 invasive breast carcinoma cases were selected and comparative study was done between CD 10 and other prognostic factors.

Inclusion Criteria

1) Patients with age from 21-85 years who were treated with surgery as first line of management.
2) Mastectomy, Modified Radical Mastectomy, Lumpectomy specimens
3) Core biopsy specimen received in the Department of Pathology.
4) Breast specimens with invasive breast carcinoma irrespective of type or grade that are diagnosed by our histopathology department.

Exclusion Criteria

1) Benign lesions
2) Other non-neoplastic lesions
3) Mastectomy specimens diagnosed as malignant tumors of stromal origin.
4) Patients who underwent chemotherapy, radiotherapy or hormonal therapy before surgery are not included in study as they will cause alterations in the CD10 expression.

Consent

Consent is not needed since tissues and tissue blocks are used for study.

4. Methodology and Techniques

380 malignant cases were analysed. Clinical and morphological details of cases including the name, age, sex, histopathology number, clinical history, tumour size, laterality, menstrual status, any treatment given, histopathological diagnosis, histological grading, lymphnode metastasis, ER, PR, HER2 neu status, CD 10 expression (among selected 100 invasive breast carcinoma cases) were recorded.

Modified radical mastectomy specimens, tru cut biopsies were collected and fixed for 12 hours in neutral buffered formalin. After fixation of MRM and Mastectomy specimens skin nipple were examined and photography of specimens were taken and specimens were sliced at an interval of 5 to 10 mm.

Tumor size was measured as greatest dimensions and additional dimensions of the invasive component with recording of the distance from the resected surgical margins. Nodes were recorded by dissecting from the breast fat

adjacent to tumor and from the axillary pad of fat. Representative bits were taken. The bits were subjected to automated tissue processing and embedded in paraffin. Around 4 µm thin thickness were obtained and stained with Eosin and Haematoxylin.

The slides were reviewed. Histological diagnosis and grading were done according to WHO classification of breast tumors.

Immunohistochemical Evaluation

Sections of 3µ thickness were cut from paraffin embedded tissue blocks. The sections were transferred to 3-aminopropyltriethoxysilane coated slide. Antigen retrieval was done by heat induced method. Mouse monoclonal antibody would bind with antigen and then detected after adding horse radish peroxidase (HRP) polymer and Diaminobenzidine substrate (DAB)-secondary antibody.

5. Interpretation and Scoring System

Antibody treated slides were analysed for presence or absence of reactivity and the percentage of cells stained by CD 10. Staining pattern was cytoplasmic or membranous positivity.

CD 10 expression interpretation

Interpretation	Description
Negative	< 10% cytoplasmic and membranous staining in stromal cells
Weak positive	10-30 % focal cytoplasmic and membranous staining of stromal cells Diffuse weak staining
Strong positive	>30 % cytoplasmic and membranous staining of stromal cells.

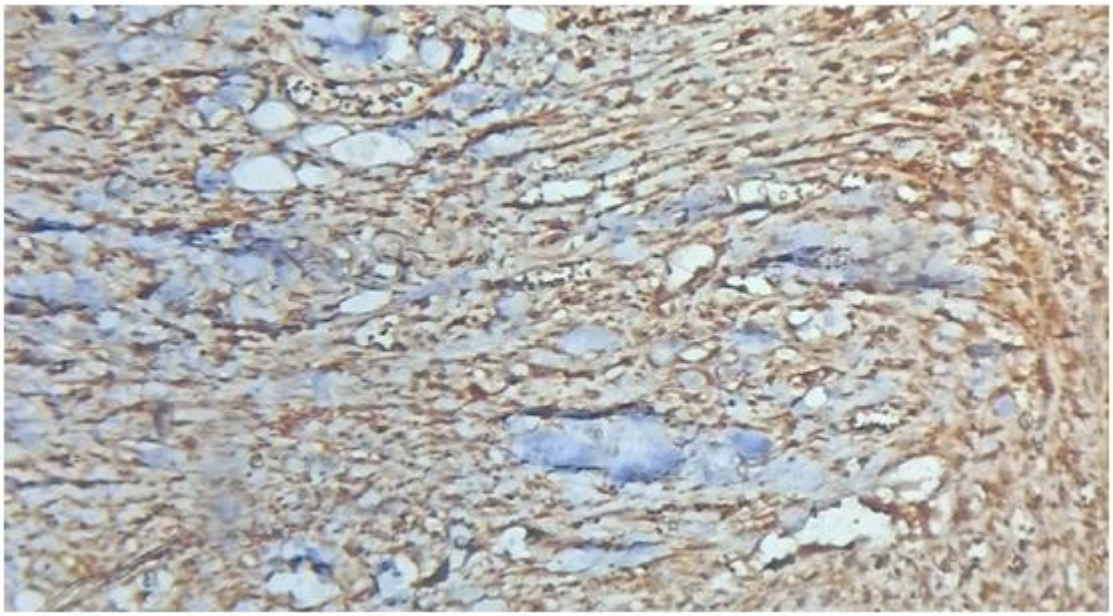

Figure 1: Invasive carcinoma NST CD 10 strong positive - >30 % stromal cells expressed it (100X)

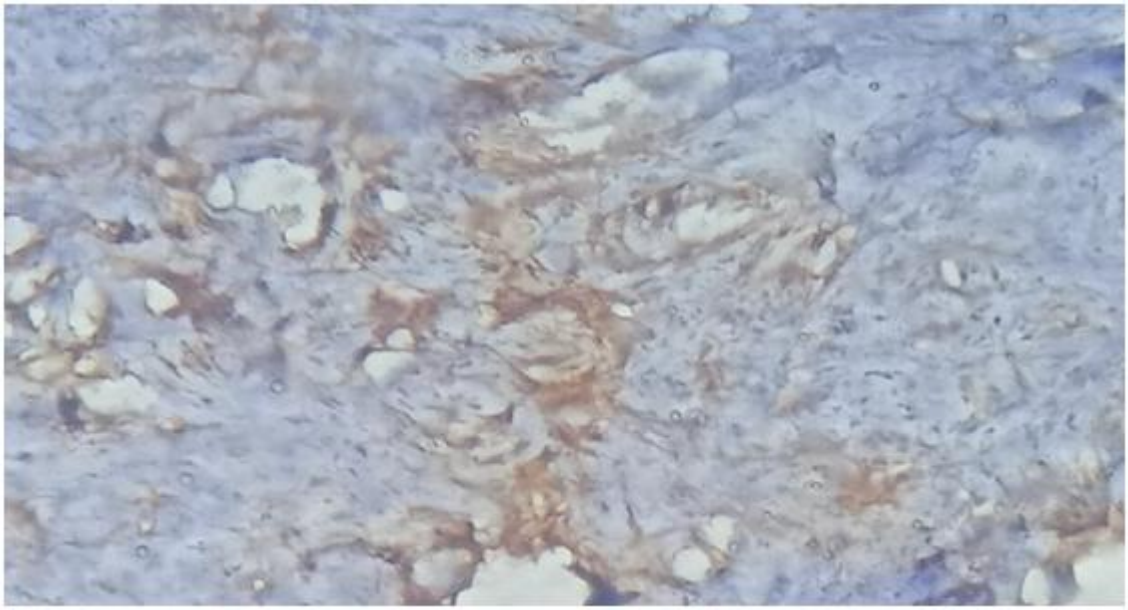

Figure 2: Invasive carcinoma NST-CD 10 weak positive (10-30% positivity, faint in intensity) - 100X

The correlation between CD 10 and various clinicopathological parameters like tumour size, nodal status, histopathological grade, ER, PR, HER 2neu status were analysed. By using Pearson correlation of coefficient and Chi square test, the strength of association was calculated.

6. Observation and Results

100 cases were selected from the malignant tumours (composed of 94 Invasive carcinoma breast of NST, 1 case of invasive breast carcinoma with medullary pattern, 1 case of invasive breast carcinoma with apocrine differentiation, 1 case of invasive papillary carcinoma, 1 case of mucinous carcinoma, 1 case of invasive lobular carcinoma and 1 case of metaplastic carcinoma).

Nottingham histologic grade distribution
Among 100 cases, Nottingham histologic grade were applied for invasive carcinoma breast NST for 51 mastectomy specimens. The number of cases under grade I were 9 (17.65%), grade 2 were 37 (72.55%) and grade 3 were 5 (9.80%).

Lymphnode staging frequency distribution
Out of 100 cases, the axillary lymphnodes were dissected and recorded from mastectomy specimens. The number of cases in which axillary lymphnodes dissected were 41. Microscopic tumour involvement of the nodes were identified and were staged according to AJCC TNM staging system for carcinoma breast. The total number of cases shown metastatic carcinomatous deposits in lymphnodes were 22.

ER, PR, HER2 neu and CD 10 immunohistochemistry were done for these 100 cases. CD 10 results were correlated with tumour size, lymphnode status, histological grading, ER, PR and HER2 expression status.

ER, PR and HER2 neu scoring was done according to CAP protocols.

Correlation of CD 10 with Tumour size
Tumour size were obtained from the mastectomy cases and from the given clinical history. The number of cases in which tumour size known were 59. On comparison between the tumour size and CD10 expression, the number of cases with pT1 category among 59 cases were 3 of which 2 cases had shown CD 10 strong positivity and 1 had shown CD 10 negativity (33.33%).

The total number of cases with tumour size 2 to 5 cm (pT2) were 28. Among these, the number of cases shown CD 10 positivity were 22 (78.57%) of which 8 cases (28.57%) shown weak CD 10 expression and 14 cases (50%) shown strong CD 10 expression. CD 10 was negative in 6 cases (21.43%).

The number of cases with pT3 were 26, among them weak positivity was seen in 3 cases (11.54%), strong CD 10 positivity was seen in 14 (53.85%) cases and CD 10 negativity seen in 9 (34.62%)cases.

The total number of cases fell under pT4 group were 2 and both of them shows strong CD 10 positivity (100%).

Most of the CD 10 expression were seen among pT4 categories that comprised of 100% strong positivity expression followed by pT2 category (78.57%) with 50% cases shown strong positive expression and 28.57% cases shown weak positivity.

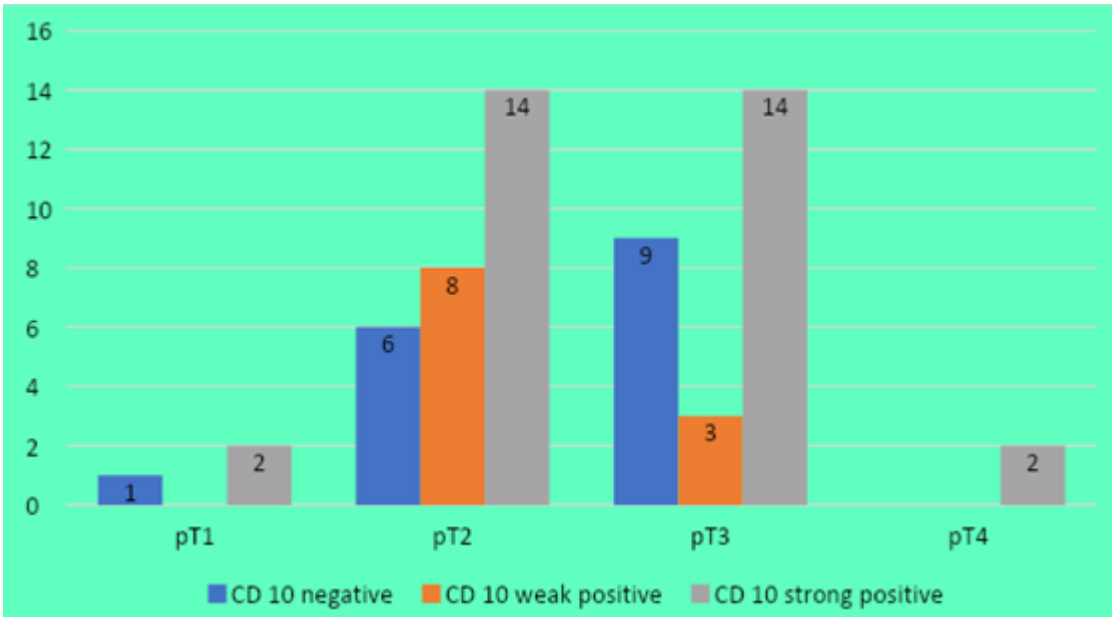

Chart 1: Correlation of CD 10 with Tumour size

These results were analysed in Pearson correlation co-efficient and were not found to be statistically significant with a p value of 0.123452.

Correlation of CD 10 with lymphnode metastasis
Out of 100 patients, 22 cases shown metastatic carcinomatous deposits in lymphnodes. On correlation between CD 10 expression and lymphnode status, 9 patients

had 1 to 3 axillary lymph nodes involvement in whom 2 patients (22.22%) shown CD 10 negativity, 3 patients (33.33%) had shown weak positive,4 patients (44.44%) had shown CD 10 strongpositivity.

8 patients had 4 to 9 lymph nodes involvement. Among them, 2 cases (25%) had CD 10 negativity, 2 cases (25%) had weak positivity in CD 10 expression and 4 cases (50%) had CD 10 strong positivity.

5 patients had ≥ 10 lymph nodes involvement, of which 1 case (20%) had shown CD 10 weak positivity and 4 cases (80%) had shown CD 10 strong positivity.

The number of cases shown no nodal involvement with reactive changes were 19 (4 cases shown CD 10 negativity, 3 cases had shown CD 10 weak positivity and 12 cases had shown CD 10 strong positivity).

Table 1: Correlation of CD 10 with lymphnode metastasis

Positive lymphnodes	Total number of cases	CD 10 negativity	CD 10 weak positivity	CD 10 strong positivity
1 to 3 (pN1)	9	2	3	4
4 to 9 (pN2)	8	2	2	4
≥ 10(pN3)	5	-	1	4
Total	22	4	6	12

The majority of cases shown CD 10 expression lie in pN3 category 5/5 (100%) all showing strong CD 10 expression, followed by pN1 category 7 out of 9 cases (77.78%) in our study.

But the intensity of CD 10 expression increases from weak positivity to strong positivity on increasing trend in lymph node staging (44.44%, 50%, 80% of strong positivity in pN1, pN2, pN3 categories respectively).

The proportion of CD 10 positivity among lymph node metastasis cases were 81.82% higher than CD 10 positivity seen in lymph node negative cases (78.95%).

These observations were analysed statistically with p valve = 0.333333 obtained through Pearson correlation co-efficient and the result was not found to be statistically significant.

CD 10 comparison with Histopathological grade

Among 100 cases, histopathological grading was done for 51 mastectomy cases. 9 cases had shown histological grade I, 37 cases shown grade II and 5 patients shown grade III. Comparison between CD 10 expression and histopathological grading was done.

Among the 9 grade I tumours, 7 cases (77.78%) had shown CD 10 negativity,1 case (11.11%) had shown CD 10 weak positivity and 1 case (11.11%) had shown CD 10 strong positivity.

In grade II group,13 cases (35.13%) shown negative expression of CD 10,10 cases (27.02%) had shown weak positive expression of CD 10 and 14 cases (37.84%) shown strong positive expression of CD 10.

Among 5 grade III cases, 1 case had shown CD 10 weak positivity (20%), 3 cases shown CD 10 strong positivity (60%) and 1 case shown negative CD 10 expression (20%).

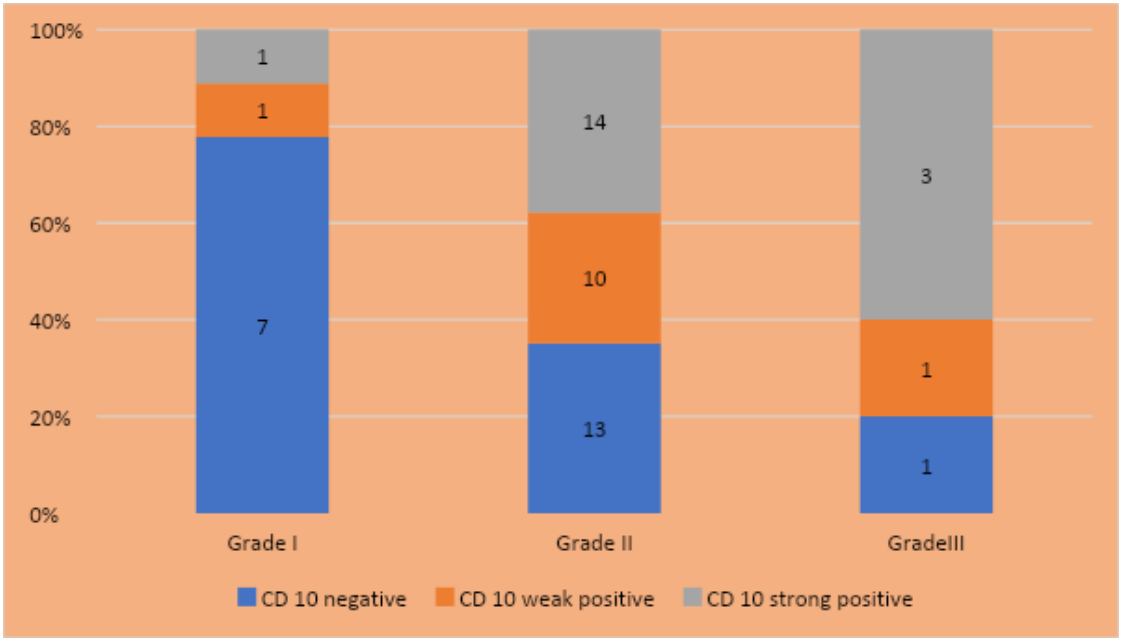

Chart 2: CD 10 comparison with Histopathological grade

CD 10 positivity was seen in highest proportion in Grade III tumours (80%) as 4 cases had shown CD 10 expression (included 20% weak positive and 60% strong positive) followed by Grade II tumours (64.86%). The chi-square statistic obtained was 6.461 and the p value was found to be statistically significant (p=0.039537). CD 10 expression was increased as the histological grade advanced.

CD 10 correlation with ER status

Out of 100 cases, the number of cases showing ER negativity were 75, of which 17 cases (22.67%) had shown CD 10 negativity, 20 cases (26.67%) had shown CD 10 weak

positivity and 38 cases (50.67%) had shown CD 10 strong positivity. The number of cases shown ER positivity were 25, of which 12 cases had shown CD 10 negativity (48%), 04 cases (16%) had shown CD 10 weak positivity and 09 cases (36%) had shown CD 10 strong positivity.

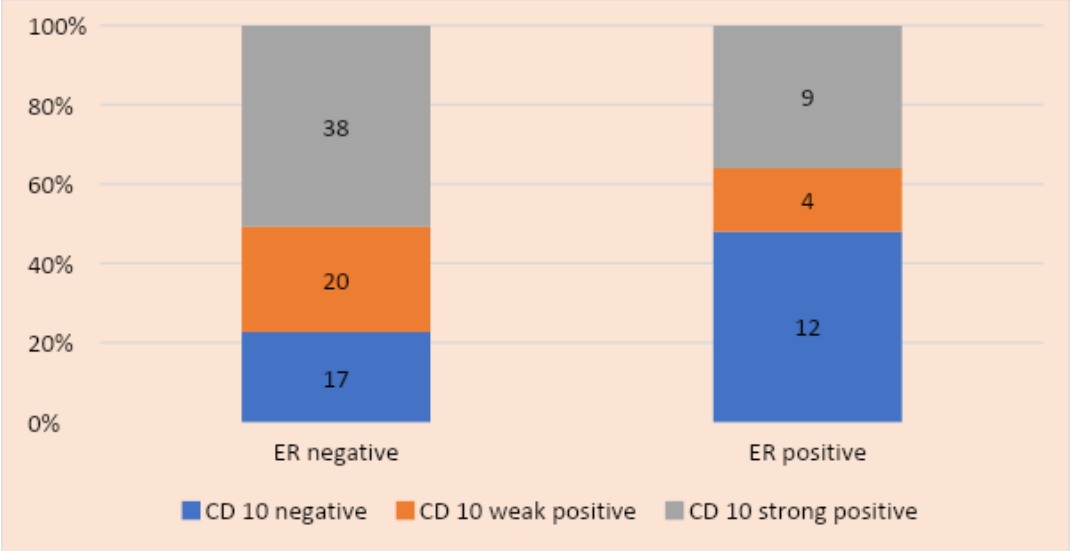

Chart 3: CD 10 correlation with ER status

A significant higher proportion of CD 10 positivity (77.33%) was seen among ER negative cases than among ER positive cases (52%). CD 10 expression was increased when the ER negativity was increased and CD 10 negativity was increased when ER positivity increases. The chi-square statistic obtained with these observations was 5.8443 with p value = 0.015628 and was found to be statistically significant with p value less than 0.05.

CD 10 comparison with PR status

Out of 100 cases, the number of cases shown PR positivity were 24 and 76 cases shown PR negativity with majority of cases in our study shown PR negativity. Out of 24 PR positive cases, 08 cases (33.33%) had shown weak positive CD 10 expression, 09 cases had shown strong positive CD 10 expression (37.50%) and 07 cases shown CD 10 negativity (29.17%). Out of 76 PR negativity cases, CD 10 negativity seen in 22 cases (28.95%), CD 10 weak positivity seen in 16 cases (21.05%) and 38 cases had shown strong positive CD 10 expression (50% cases).

Table 2: CD 10 comparison with PR status

Progesteron e receptor status	Total cases	CD 10 negativity	CD 10 weak positivity	CD 10 strong positivity
Negative	76	22	16	38
Positive	24	07	08	09

In this observation the proportion of cases showing CD 10 positivity among PR negative cases were 71.05% little higher than CD 10 positivity among PR positive cases (70.83%). The chi-square statistic obtained was 0.0004 with p value = 0.983532 and was not found to be statistically significant.

Comparison of CD 10 with HER2 neu status

The total number of cases shown HER2 neu negativity were 56. Among those cases, the number of cases had shown CD 10 negativity was 18 (32.14%) and CD 10 positivity were 38 (included 12 weak positive cases and 26 strong positive cases) with proportion of 67.85%. The number of cases shown HER2 neu positivity were 44, among them 11 cases had shown CD 10 negativity (25%), 12 cases shown weak positivity (27.27%) and 21 cases shown CD 10 strong positivity(47.72%).

The proportion of cases with CD 10 positivity among HER2 neu positive cases were 75%, higher than that seen in those with CD 10 negativity (67.86%) and CD 10 negativity was more in HER2 neu negative cases (32.14%) than HER2 neu positive cases(25%).

With this observation, chi-square test was applied and the chi-square statistic was found to be 0.6106 with p value= 0.434577 and were not statistically significant.

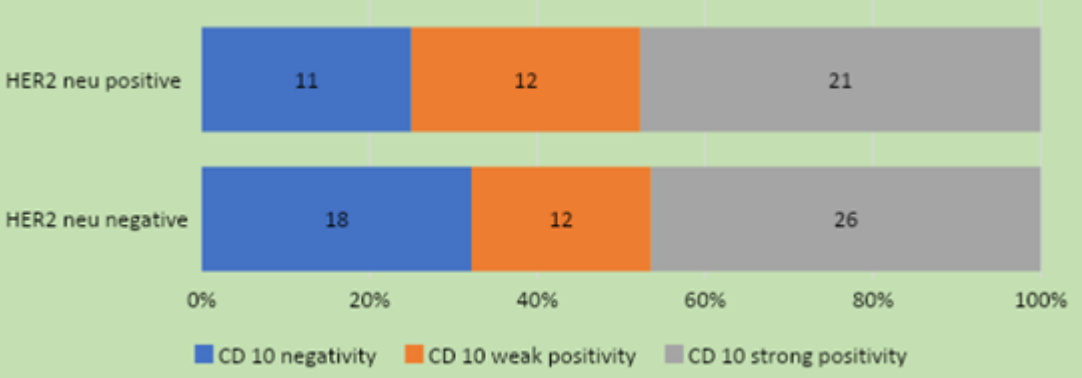

Chart 4: Comparison of CD 10 with HER2 neu status

7. Discussion

Major public health problem for women is breast carcinoma. Invasive carcinoma breast has been reported most frequently in our Department of Pathology, Madurai medical college.

Stromal cell proliferation is a feature of invasion of cancer and metastasis. The clinical significance of stromal CD 10 expression in invasive ductal carcinoma has been addressed. In majority of normal breast tissue, there is no expression of CD 10. CD 10 negativity in myoepithelial cells are seen in early stage of breast carcinoma with its progression from DCIS and metastasis, followed by abnormal expression in the stroma in later stages of invasive breast carcinomas. Conversion of fibroblasts to myofibroblasts trans differentiation also played a role in invasion of cancer cells.

Since CD 10 is a matrix metalloproteinase, it facilitates the degradation of matrix and its invasiveness. The accumulation of CD 10 cleaved peptides inhibit the differentiation of epithelial cells and blockage in PTEN functions with apoptosis inhibition lead to cell proliferation and angiogenesis through Akt pathway. In general, CD 10 positivity is associated with diminished metastasis free survival. Knowledge regarding the stromal role in breast cancer development and progression will aid us in identifying therapeutic targets and new prognostic markers.

The scoring system of CD 10 expression used in our study divided the cases into negative, weak positive and strong positive based on the study by Makretsov et al[3]. Masaki et al suggested CD 10 expression to be positive when more than 10% stromal cells around tumour cells expressed it[112].

Since tumour size and status of lymphnode are the determinants of staging of breast tumours, these also considered as specific prognostic factors for correlating with CD10 expression.

CD 10 comparison with Tumour size
In our study, among 100 cases, the proportion of cases with CD 10 positivity were higher in pT4 parameter (100% cases expressed it) of TNM staging system followed by pT2 (78.57%) and these observations were not found to be statistically significant, p= 0.123452.

A study done by Vandana Puri et al with 50 cases had observed most of cases (21/50,42%) were in T4 category, followed by T2 category with 15 out of 50 cases (30%). CD 10 strong positivity increased from 50% in T2 category to 64.71% in T4 category, but there were no statistical significant correlation with p =0.558[4].

Comparison of CD 10 expression and lymphnode status
In our study, among 100 cases, a significant proportion of cases (100%) had shown CD 10 strong positivity among N3 parameter group of TNM staging, followed by N1 parameter stage group and these results were not found to be statistically significant with p valve = 0.333333.

Azza M.Rizk et al conducted a study included 60 invasive carcinoma NST types, of which 46 cases (76.67%) had shown positive lymph node metastasis,14 cases(23.33%) shown negative lymph node metastasis. CD 10 positivity was seen in 38 cases among lymph node metastasis cases (included 9 strong positivity and 29 weak positivity). Around 11 negative lymph node metastasis cases had shown CD 10 positivity (1 strong positivity and 10 weak positivity). Even though higher proportions of CD 10 positivity were seen in lymph node positive cases (82.61%) than lymph node negative cases (78.57%), the results were not found to be statistically significant with p value = 0.547[5].

Comparative study of CD 10 with Histopathological grading
In our study, majority of cases were of grade II (37/51 cases, 72.55%). The higher proportion of grade III cases (80%) shown CD 10 positivity (included 20% weak positive and 60% strong positive cases), followed by Grade II tumours (64.86%). When these observations were entered in chi-square test, it was found out to have a significant correlation with p=0.039537. Stronger expression of CD 10 was seen in grade III and the proportion of CD 10 positivity increases when the grade increases, indicating it is a prognostic marker of aggressive behavior.

Emad Sadak et al in their study with 97 cases of invasive ductal carcinoma, 15 cases (42.9%) among grade 1-2 category and 20 cases (57.1%) among grade III category shown CD 10 positivity. 58 cases (93.5%) in grade 1-2 category and 4 cases (6.5%) among grade III category had shown CD 10 negativity. These observations were found to be significant statistically with p value <0.001%[6] as seen in our present study.

CD 10 correlation with Estrogen receptor status
Out of 100 cases in our study, 75 cases shown ER negativity, of which 77.33% cases shown CD 10 positivity. Among the

ER positive cases, 52% cases expressed CD 10 positivity. So a higher proportion of CD 10 positivity were seen among ER negative cases. These observations were found to have a statistically significant inverse correlation with p value=0.015628.

Studies with significant inverse correlation between CD 10 and ER status

Study name	ER status	CD 10 expression	p value
Ali Taghizadeh- Ker mani et al[111]	ER negative-36 cases ER positive-64 cases	83.33% cases show positivity among ER negative group and 53.12% positivity among ER positive group	0.003
Emad Sadaka et al[117]	ER negative-25 cases ER positive-72 cases	21 cases show positivity among ER negative group and 14 positivity among ER positive group	<0.001%

CD 10 with Progesterone receptor status

In our study, among 100 cases 76 cases shown PR negativity. CD 10 positivity were seen in 71.05% of PR negative cases and 70.83% among PR positive cases. A little higher expression of CD 10 was seen in PR negative group than PR positive group. These observations were not found to be statistically significant with p value= 0.983532.

Ashish Nitin Dhande et al in their study with 60 cases, the number of cases showing PR negativity were 40 and PR positive cases were 20. Out of 40 PR negative cases, 33 shown CD 10 positivity and out of 20 PR positive cases,14 shown CD 10 positivity. Even though higher number of cases exhibiting CD 10 positivity among PR negative cases as like of our study, these were not found to be significant statistically with p value= 0.438[7].

CD 10 analysis with HER2 neu status

In our study, the number of cases exhibited HER2 neu positivity were 44 and 56 cases shown HER2 neu negativity.75% of cases shown CD 10 positivity among HER2 neu positive group and 67.86% of cases shown CD 10 positivity among HER2 neu negative group. But statistically, on correlation with CD 10 positivity with HER2 neu status, it was not found to be significant with p value= 0.434577.

Azza M.Rizk et al in their study with 60 Egyptian invasive carcinoma NST cases,49 shown HER2 neu negative and 11 cases shown HER2 neu positive. Among HER2 neu positive cases, 10 cases shown CD 10 positivity and among HER2neu negative cases, 39 cases exhibited CD 10 positivity. But these results were not found to be significant statistically as like our case with p value =0.432[119].

8. Conclusion

- Our present study highlights stromal CD 10 role in prediction of tumour response and progression
- Presence of CD 10 positive expression in stromal cells correlate with worse prognostic factors like higher histological grade and ER negativity as seen in our study.
- CD 10, a novel stromal marker to be used as an independent prognostic marker along with ER, PR, HER2 neu Immunohistochemistry before giving chemotherapy.
- Since it prevents the cancer stem cell differentiation and may cause recurrence of carcinoma, targeted therapy to be developed against CD 10 that may give promising regards.
- CD 10 being a metalloprotease, can cleave CPI-0004Na and peptide prodrugs like sAIAL-Dox creating a higher potency than Dox alone, thereby will be useful in determining the line of treatment of patients.
- As the drug Doxorubicin is cleaved by CD 10 causing chemoresistance, inhibiting its activity will lead to increased chemotherapy response for the common morbid condition among females **"THE INVASIVE BREAST CARCINOMA".**

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