Stromal Expression of CD10 in invasive Ductal Carcinoma and its relationship with ER, PR and HER2-neu Status

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Abstract

Background: Ductal carcinoma is a common type of breast cancer that starts in cells of milk ducts and can enhance the risk of developing invasive breast cancer in the long run. The study aimed to evaluate the stromal expression of CD10 in Invasive Ductal Carcinoma and its relationship with ER, PR, and HER-2 status.

Methods: This cross-sectional study was carried out in the Department of Pathology, Rajshahi Medical College, Rajshahi, from July 2017 to December 2018, to evaluate the expression of stromal CD10 in invasive breast carcinoma and to correlate with ER, PR, HER 2/neu status in mastectomy or lumpectomy specimen. A total of 50 cases of mastectomy or lumpectomy specimens will be taken that were received in the Department. Samples were selected by the purposive sampling technique.

Results: In the present study, out of total 50 cases, most of the study subjects that is 17 (34%) belonged to the age group of 51-60 years. Estrogen receptor were positive in 29(58.0%) cases, progesterone receptor was positive in 16(32.0%) cases. The association between estrogen receptor status with stromal CD10, all the 16 (100.0%) CD 10 strong positive cases were negative for estrogen receptor. In CD10 negative group, almost all 28 (96.6%) cases were positive for estrogen receptor. The difference was statistically significant ($p<0.05$).

Conclusion: Substantial knowledge regarding stromal contribution to cancer progression could help researchers to identify particular signals which could lead to the revelation of new therapeutic targets in the future.

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Keywords: Ductal carcinoma, CD10, Estrogen receptor, Progesterone receptor, Her-2, Invasive

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Introduction

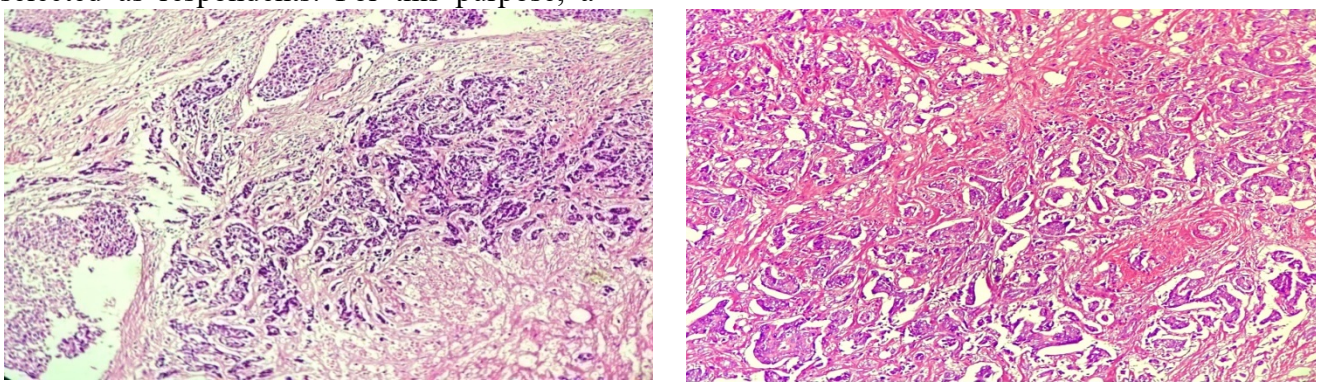
Breast cancer is the second greatest cause of cancer death in the world, after lung cancer,[1] and the most often diagnosed cancer in women in most of the countries in the world.[2] More than half (52.9%) of 1.67 million new breast cancer cases were detected in developing countries in 2012, according to GLOBOCAN estimates.[3] Breast cancer incidence has climbed by more than 20% since the 2008 estimates, while mortality has increased by 14%.[2] It will affect more than 2 million people worldwide every year by 2030.[4] Despite the fact that the incidence has been rising in most parts of the world, there are significant disparities between rich and poor countries. In industrialized countries, early detection and administration of target-based therapy for breast cancer improve patient survival.[5] As a result, while incidence rates remain highest in more developed regions,[4] mortality rates in less developed countries are significantly higher due to a lack of early detection and treatment facilities. For example, whereas black women have a slightly lower overall breast cancer incidence rate than white women, black women have a 42 percent higher breast cancer death rate.[1] The mortality disparity is likely due to a combination of biologic and nonbiologic factors, such as disparities in diagnosis stage, co morbidities, tumor features, as well as therapy access, adherence, and response.[6] According to GLOBOCAN estimates based on extrapolation of Indian data, 14,836 new cases of breast cancer were reported in Bangladesh in 2012, with among them 7,142 women were died.[3] Breast cancer kills 8,396 people each year, according to the International Agency for Research on Cancer (IARC).

Early detection relies heavily on self-breast examination and clinical breast examination. The triple assessment test, which combines clinical examination, radiographic imaging (mammography, ultrasonography), and pathology, is currently the gold standard for diagnosing all palpable breast masses. They have a combined sensitivity of 99 percent.[7] Stromal markers, on the other hand, are emerging as novel markers in predicting the prognosis of invasive breast cancer and have yet to be thoroughly investigated.[8] A greater knowledge of the stromal contribution to cancer progression will lead to the identification of particular signals that enhance tumor cell growth, dedifferentiation, invasion, and ectopic survival, as well as new therapeutic targets for future treatment.[9] Chemical mediators between tumor cells and stromal cells have a role in tumor epithelial development.[10] CD10 reduction in myoepithelial cells and CD10 expression in stromal cells, which is a typical feature of epithelial to mesenchymal transition (EMT), is linked to aggressive behavior in invasive ductal carcinoma of the breast.[11] CD10 has been identified as a marker of stem-like or biopotent progenitor breast cells in recent in vitro investigations.[12] Because stromal CD10 expression and changes with chemotherapy may be prognostic, it should be reported in breast cancer patients before and after treatment.[13] The treatment response can be assessed by measuring stromal CD10 markers before and after chemotherapy.[14] CD10 appears to be a possible target for novel cancer therapy, according to some preliminary research. Some study [15] discovered a strong correlation between recurrence status and time to recurrence and stromal CD10 expression. ER, PR positivity has been linked to low-grade breast cancer with a better prognosis in several studies.[16] Her-2/neu, on the other hand, has been linked to tumors that are extremely aggressive and of high grade.[17] As a result, determining ER, PR, and HER-2/neu status has clinical significance and is commonly utilized in pathological diagnosis.[16] CD10 immunohistochemistry in invasive breast cancer and its connection with ER, PR, and

HER2/neu has been shown to be able to predict invasive and metastatic tumor potency, which can help with treatment protocol selection, prognosis, and patient survival. The study aimed to evaluate the stromal expression of CD10 in Invasive Ductal Carcinoma and Its relationship with ER, PR, and HER-2 status.

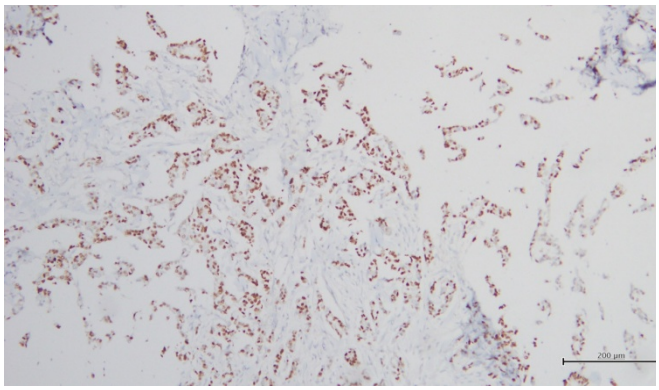
Method

This cross-sectional study was carried out in the Department of Pathology, Rajshahi Medical College, Rajshahi, from July 2017 to December 2018, to evaluate the expression of stromal CD10 in invasive breast carcinoma and to correlate with ER, PR, HER 2/neu status in mastectomy specimen. Data were collected from the Department of Surgery, Rajshahi Medical College Hospital. Females of different age groups having a different breast lump suspicious for malignancy admitted to the Surgery Department in Rajshahi Medical College Hospital were selected as respondents. For this purpose, a total of 50 cases of mastectomy or lumpectomy specimens were taken that were received in the Department. Samples were selected by the purposive sampling technique. For each case, five sections were obtained. One for routine H and E staining (Figure 1) and the other for immunohistochemical analysis with ER, PR, HER2/neu, and CD0 immune marker (Figure 2). Patients with duct cell carcinoma (NOS) with histological confirmation were included in this study. Benign breast disease, previously diagnosed cases and having therapy, cystic lesion and microcalcification without definite lump, patients suffering from uncontrolled DM, severe hypertension, IHD, respiratory failure and coagulopathy, and inadequate sample were excluded from the study. Priorto the commencement of this study, the thesis protocol was submitted to the Institutional Review Board (IRB) of RMC, Rajshahi for approval and was approved.

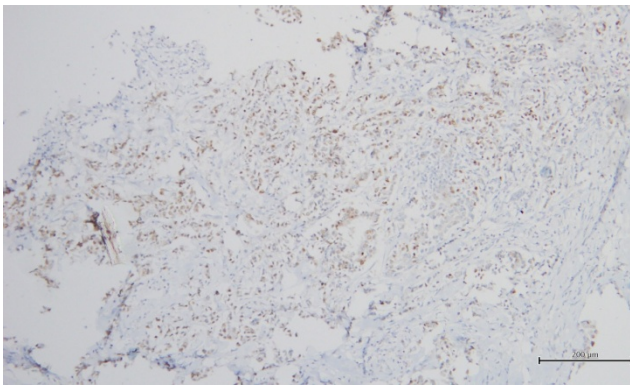


A B

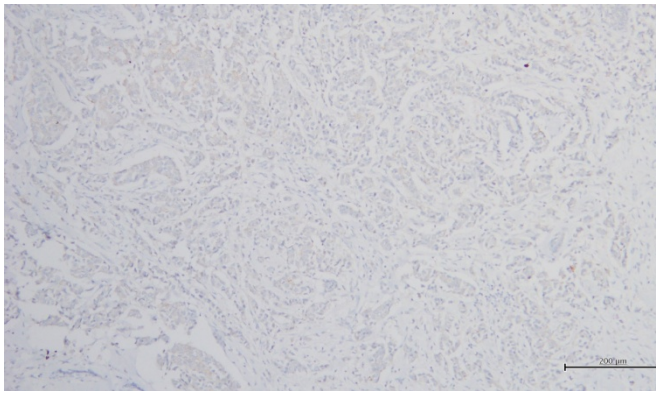
Figure 1. Duct Cell Carcinoma Breast Grade 2 (A 10x B 40x)



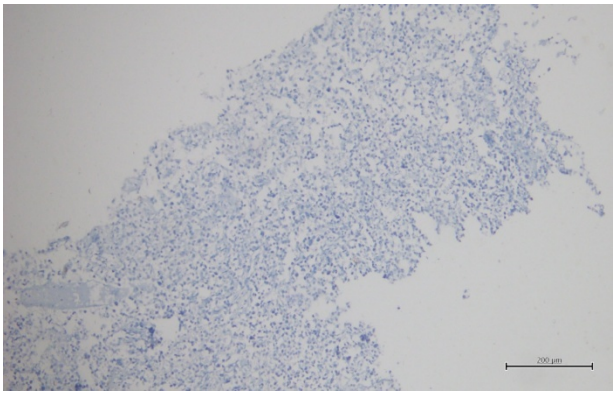
ER Receptor (10x) positive



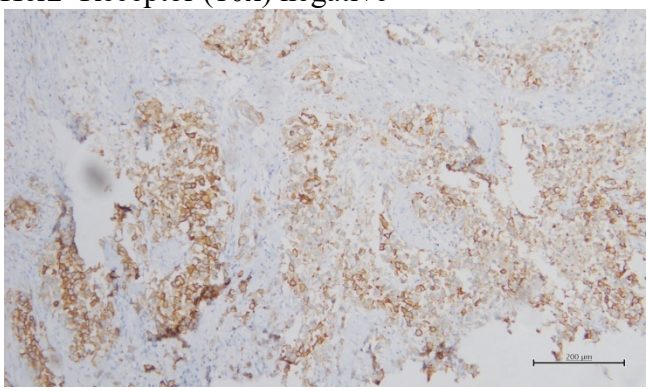
PR Receptor (10x)positive



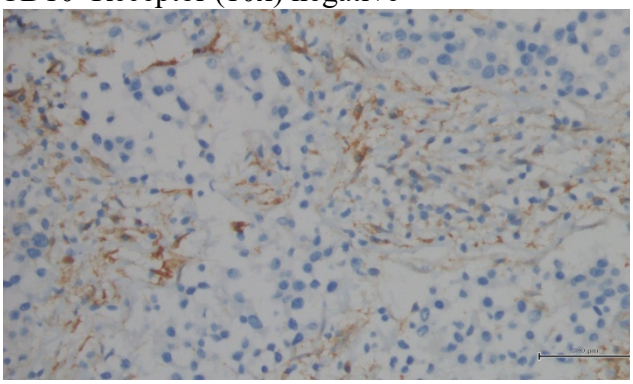
Her2 Receptor (10x) negative



CD10 Receptor (10x) negative



Her2 Receptor (10x) positive



CD10 Receptor (10x) positive

Figure 2. Photomicrograph of immunohistochemistry

Sample processing and forwarding
Collected tissue will be kept in 10% formalin in a properly labeled container assigned with a laboratory number and will be fixed for 8 to 48 hours. In the laboratory, tissue processing, paraffin embedding, sectioning of the paraffin blocks, H & E staining will be done according to the standard protocol and will be assessed for histological diagnosis.

Routine histopathological examination
Histopathological type of tumor (according to WHO classification of breast tumor) and grading (Nottingham modification of the Bloom -Richardson Grading System) of all the cases were done and recorded.

Immunohistochemistry for ER, PR and Her2/neu
Immunohistochemistry of all selected cases for ER, PR, HER-2/neu expression were performed using Dako Autostainer Plus and rabbit monoclonal antibody at the Immunohistochemistry Laboratory, Armed Forces Institute of Pathology, Dhaka Cantonment, Dhaka. Interpretation of the results were compared with positive controls.

IHC of ER, PR were scored according Allred score:[18]

Score for proportion of positive staining cells	Score for staining intensity
0= No nuclear staining.	0= No Staining of any nuclei.
1= 1 % nuclear staining.	1= Weak Staining.
2=1-10 % nuclear staining.	2= Intermediate Staining.
3= 10-33 %nuclear staining.	3= Strong Staining.
4= 33-66 % nuclear staining.	
5= 66-100% nuclear staining.	

Sum of proportion score and intensity score

Total score interpretation
Score 0 - 2 Negative
Score 3 – 8 Positive

Interpretation of Her2/neu status according to ASCO score:[19]
0=No staining observed or membrane staining is observed in less than 10% of tumor cells.
1+=A faint/barely perceptible membrane staining is detected in more than 10% of tumor cells.
2+= A weak to moderate complete membrane staining is seen in > 10% of tumor cells (weakly positive).
3+= A strong complete membrane staining is seen in > 30% of tumor cells (strongly positive).

Results

Estrogen receptor and Progesterone receptor

Out of 50 respondents 29(58.0%) cases were estrogen receptor positive. Progesterone receptor was found to be positive in 16(32.0%) cases.

Association of estrogen receptor (ER) status with stromalCD10

All strong positive CD10 were ER-negative. One (20.0%) weak positive CD10 was ER-positive. Out of 29 negative CD10 cases, 28 (96.6%) were ER-positive. The difference was statistically significant ($p<0.05$) (Table II).

Table I: Association between estrogen receptor with stromal CD10 (n=50)

Estrogen receptor	Stromal CD 10 n (%)			p-value
	Strong positive (n=16)	Weak positive (n=5)	Negative (n=29)	
Positive	0 (0.0)	1 (20.0)	28 (96.6)	<0.001[s]
Negative	16 (100.0)	4 (80.0)	1 (3.4)	

s = significant

Chi-square test was done to measure the level of significance

Association of progesterone receptor status with stromalCD10

All strong positive CD10 was negative for progesterone receptor. One (20.0%) weak positive CD10 was progesterone receptor positive. Out of 29 negative CD10 cases, 15 (51.7%) were progesterone receptor positive. The difference was statistically significant ($p<0.05$).

Table II: Association between progesterone receptor with stromal CD10 (n=50)

Progesterone receptor	Stromal CD 10 n (%)			p-value
	Strong positive (n=16)	Weak positive (n=5)	Negative (n=29)	
Positive	0 (0.0)	1 (20.0)	15 (51.7)	0.001[s]
Negative	16 (100.0)	4 (80.0)	14 (48.3)	

s = significant

Chi-square test was done to measure the level of significance

Association of Her-2/neu status with stromal CD10

Of total 50 study cases, 16 were Her-2/neu positive. Of these, 13 cases were strong positive for CD10, 3 cases were weak positive for CD10. In the weak positive group (5 cases) of Her-2/neu, only 4 cases were in CD10 strong positive group. The difference was statistically significant ($p>0.05$) among these three groups and also found Kappa value 0.712 (Table V).

Table III: Association between Her-2/neu status with stromal CD10 (n=50)

Her-2/neu status	Stromal CD 10 n (%)			p-value
	Strong positive (n=16)	Weak positive (n=5)	Negative (n=29)	
Strong positive	13 (81.3)	4 (80.0)	0 (0.0)	
Weak positive	3 (18.8)	1 (20.0)	1 (3.4)	<0.001[s]
Negative	0 (0.0)	0 (0.0)	28 (96.6)	

s = significant
P value reached from Chi-square test

Discussion

In this study out of total 50 cases, most (34.0%) of the study subjects belonged to the age group of 51-60 years followed by 32.0% belonged to 41 -50 years, 24.0% belonged to 31- 40 years, 6.0% belonged to >60 years and 4.0% belonged to ≤30. The mean age was 47.0±9.6 years. In this current study, it was observed that all the 16 (100.0%) CD 10 strong positive cases were negative for estrogen receptor. In CD10 negative group, almost all (96.6%) cases were positive for estrogen receptor. The difference was statistically significant ($p<0.05$). Similarly, some studies showed statistically significant association between strong CD10 staining and ER negativity.[20,22,11,9] A similar association was also observed by some other studies.[14,21] In this series, 16 cases were Her-2/neu positive. Of these, 13 cases were strong positive for CD10, 3 cases were weak positive for CD10. In the weak positive group (5 cases) of Her-2/neu, only 4 cases were in CD10 strong positive group. The difference was statistically significant ($p>0.05$) among these three groups and also found Kappa value of 0.712. A study revealed an association of stromal CD10 with Her-2/new in breast carcinoma. [22] Some other studies revealed a statistically significant correlation between stromal CD10 expression and HER2/neu status.[5,15] In the development, progression, hormonal expression along with response to chemotherapy in breast cancer, an emergent role has been played by stroma. Stromal markers have necessitated for the study of breast cancer. CD10, a novel stromal marker leads a crucial preface in case of normal breast involution, development, and progression of breast carcinoma. CD10 positive stromal signature also carried prognostic value in the HER2 positive breast cancer.[23] A possible explanation, offered by Desmedt C et al (2012) and Cabioglu N et al (2005) is that positive stromal expression of CD10 is associated with increased expression of CXCL12 which causes trans-activation of Her2, leading to increased levels of Her2.[23,24] This study also showed a significant association between CD10 positivity and Her2 over expression. All these points to the fact that stroma plays an important role in breast cancer progression and prognostification, and in coming days new markers such as CD10, TGF-β, SPARC, integrins, and laminins are to be used for better prognostification of breast cancer. In this study CD10 expression correlated strongly with well-established prognostic markers HER2-neu positivity, ER/PR negativity thus indicating, CD10 can be used as an independent marker indicating poor prognosis. It can be used as a target for the development of novel therapies. Few cohort studies established that strong CD 10 expression was associated with poor disease-free survival rate.[11]

Conclusion

This study was undertaken to detect the expression of stromal CD10 in invasive breast carcinoma in mastectomy specimen. ER

status and Her2 status showed a significant relationship with stromal CD10 indicating; CD10 can be used as an independent marker indicating poor prognosis.

Limitations

Postoperative diagnosis along with a small sample size was the main limitation of the present study. Therefore in the future, further study may be undertaken by preoperative diagnosis (core needle Biopsy) with a large sample size.

Recommendation

Further study is needed to elucidate the relationship between the disease-free survival rate or recurrence rate and stromal CD10 expression.

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