Evaluation of Stromal CD10 Expression and its Correlation with other Clinicopathological Factors in Invasive Breast Carcinoma

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Abstract

Background: Expression of stromal CD10 was considerably remarked with metastasis of lymph node and higher tumor grade. CD10 is a useful independent prognostic indicator which is necessary to include in routine histopathology report

Objective: The intent of the study was to investigate the expression of stromal CD10 in invasive breast carcinoma.

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

Result: In the present study, out of 50 cases stromal CD 10 was found to be strong positive in 13(36.1%), weak positive in 9(25%) and negative in 14(38.9%) cases. Regarding the association between tumor grade with stromal CD10 it was observed that 9 (56.3%) out of total 16 CD 10 strong positive cases, 3 (60.0%) out of five CD10 weak positive and 26 (89.7%) out of 29 CD 10 negative were grade I tumor. Seven (43.8%) were grade II tumor in the strong positive group, 2 (40.0%) in weak positive group and none in negative group. In this series it was observed that maximum 38 (76.0%) cases were grade II tumors followed by 09 (18.0%) cases were grade I and 03 (6.0%) cases were grade III tumor. The difference was statistically significant ($p<0.05$) between tumor grade with stromal CD 10 expression.

Conclusion: This study was undertaken to detect the expression of stromal CD10 in invasive breast carcinoma in mastectomy specimen. Tumor grade was significantly ($p<0.05$) associated with stromal CD 10.

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Keywords: Stromal, CD10, Carcinoma

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Introduction

After lung cancer, breast cancer is the second leading cause of cancer death around the world.[1] It is the most often diagnosed cancer among women in more than 145 countries worldwide.[2] More than half (52.9%) of 1.67 million new breast cancer cases were diagnosed in developing countries based on GLOBOCAN estimation in 2012.[3] Since the 2008 estimates, breast cancer incidence has increased by more than 20%, while mortality has increased by 14%.[2] Breast cancer patient will be more than 2 million every year by the year 2030 and it will be a global burden.[4] Early detection may improve survival of patients in developed countries.[5] As a result, incidence rates remain highest in more developed regions,[4] but mortality is relatively much higher in less developed countries due to a lack of early detection and access to treatment facilities. As for example, although the overall breast cancer incidence rate is slightly lower in black women than in white women, the breast cancer death rate is 42% higher in blacks than in whites.[6] The mortality difference likely reflects a combination of biologic and non-biologic factors, including differences in stage at diagnosis, co morbidities, tumor characteristics as well as access, adherence and response to treatments.[7] Based on the extrapolation of Indian data, GLOBOCAN estimates that, in Bangladesh, 14,836 new breast cancer cases were diagnosed in 2012 and among them 7,142 women died in the same year.[3] Currently triple assessment test, such as clinical examination, radiological imaging and Pathology used as gold standard in diagnosing all palpable breast lumps.[8] To facilitate better treatment outcome, an attempt to understand the unique biologic characteristics has been realized now a days.[7] Stromal markers CD10 is a zinc-dependent peptidase (metalloproteinase), are now coming forth as prime indicator in examining the prognosis of invasive breast cancer and have not been studied extensively till date. A clear understanding of stromal contribution to cancer progression which can identify definite signals that promote growth, differentiation, invasion, and ectopic survival of tumor cells and eventually result in the identification of new therapeutic targets for future analysis.[9] In incursive/ invasive Ductal carcinoma of breast, CD10 expression increased in stromal cells and loss in myoepithelial cell which is a basic feature of epithelial to mesenchymal transition (EMT), which lead to aggressive behavior of individual.[10] More recent in-vitro studies have defined CD10 as a marker of stem like or bio-potent progenitor breast cells.[11]

Objectives

The present study aims to assess the frequency of stromal CD10 expression in invasive breast carcinoma and evaluate its prognostic significance and correlation with other clincopathological factors like patients' age, tumor grade and LN status

Method

This cross sectional study was carried out in the department of Pathology, Rajshahi Medical College, Rajshahi, during July 2017 to December 2018, to evaluate the expression of stromal CD10 in invasive breast carcinoma and also to analyze the relationship between stromal CD10 expression and common clincopathological parameter (patient age, tumor grade, tumor size and lymph node status). Data were collected from Department of Surgery, Rajshahi Medical College Hospital. Females of different age groups having a different breast lump suspicious for malignancy admitted to Surgery Department in Rajshahi Medical College Hospital were selected as respondents. For this purpose, a total of 50 cases of mastectomy or lumpectomy specimen were taken that were received in the Department of Pathology. Samples were selected by purposive sampling

technique. For each case, five sections were obtained. One was for routine H and E staining and other was for Immunohistochemical analysis with ER, PR, Her2/neu and CD10 immune marker. 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 micro calcification without definite lump, patients suffering from uncontrolled DM, severe hypertension, IHD, respiratory failure and coagulopathy and inadequate sample were excluded from the study. Prior to the commencement of this study the thesis protocol was submitted to the Institutional Review Board (IRB) of RMC, Rajshahi for approval and it was approved.

Sample Processing and Forwarding

Collected tissue were kept in 10% buffered 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 (Figure 1).

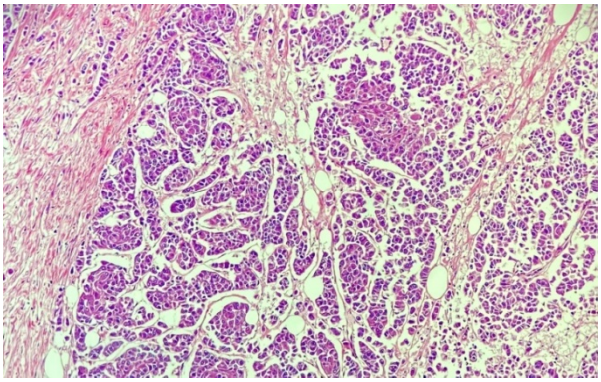

Figure 1. Duct cell carcinoma of breast (x10); Grade-2

Immunohistochemistry for stromal CD 10

CD10 expression in the stroma (both in stromal cells and extracellular matrix) was assessed by immunohistochemistry (Figure 2). From paraffin-embedded blocks, 5-micrometer thick sections were cut, deparaffinized with xylene and rehydrated through a graded series of alcohol. Antigen retrieval was done by water bath .Then the sections were stained with Monoclonal Mouse Anti-Human CD10, Code M7308, used at a dilution 1:80 DAKO EnVision TM FLEX, High pH Detection system, peroxidase/ DAB+, Rabbit/Mouse.

Section of normal vermiform appendix was taken as positive control.

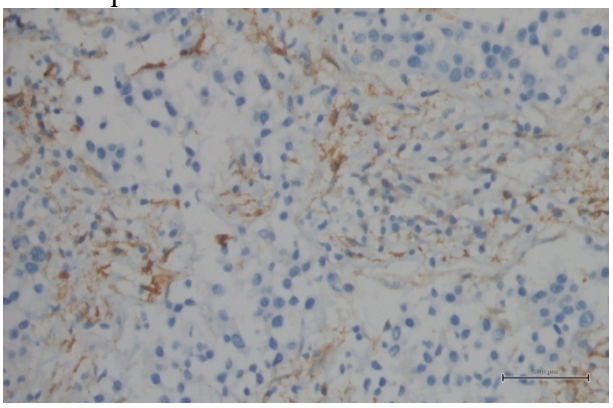

Figure 2. CD 10 receptor (x10) Olympus BX 51

Scoring for CD10[9]

CD10 expression in the tumor stroma (in both stromal cells and extracellular matrix) was scored semiquantitatively as:

• Negative: No staining

• Weak: Either diffuse weak staining or weak or strong focal staining in less than 30% of the stromal cells or extracellular matrix.

• Strong:Strong staining in 30% or more of the stromal cells or extracellular matrix

Results

Age distribution

Out of total 50 cases, most 17(34.0%) of the study subjects belonged to the age group of 51-60 years followed by 16(32.0%) belonged to 41 -50 years, 12(24.0%) belonged to 31- 40 years, 3(6.0%) belonged to >60 years and 2(4.0%) belonged to ≤30. The mean age was 47.0±9.6 years (Table I).

Table I: Distribution of cases according to age (n=50)

Age (years)	Number of patients	Percentage
≤30	2	4.0
31 – 40	12	24.0
41 – 50	16	32.0
51 – 60	17	34.0
>60	3	6.0

Mean±SD = 47.0± 9.6
Range (min-max) = 25-70

Laterality

Majority 36(72.0%) of the tumors was located on the left side and rest of 14(28.0%) the tumors were on the right side

Size of the Tumors

Tumor size was ≤3.0 cm in 26(52.0%) patients, 3.1-5.0 cm in 11(22.0%) patients and >5 cm in 13(26.0%) patients. Mean tumor size was 4.0±2.21 cm.

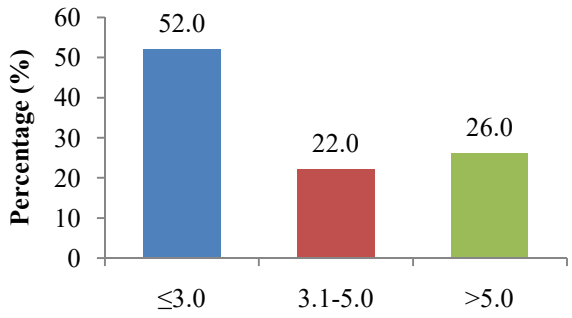

Figure 3. Bar diagram of cases according to tumor size

Tumor Grade

Maximum 38(76.0%) cases were grade II tumors followed by 9(18.0%) cases were grade III and 3(6.0%) cases were grade-I tumor (Table II).

Table II: Distribution of cases according to tumor grade (n=50)

Tumor grade	Frequency (n)	Percentage (%)
I	3	6.0
II	38	76.0
III	9	18.0

Estrogen receptor and Progesterone receptor

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

Table III: Distribution of the study cases by Estrogen receptor and progesterone receptor status (n=50)

Receptors	Positive n (%)	Negative n (%)
Estrogen receptor (ER)	29 (58.0)	21 (42.0)
Progesterone receptor (PR)	16 (32.0)	34 (68.0)

Stromal CD10 distribution

Stromal CD 10 was found to be strong positive in 16 (32.0%) cases, weak positive in 5 (10.0%) cases and negative in 29 (58.0%) cases (Table IV).

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

Progesterone Receptor	Stromal CD 10			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

LN status
LN was found positive in 21(42.0%) cases

Stromal CD 10 distribution
Stromal CD 10 was found to be strong positive in 16 (32.0%) cases, weak positive in 5 (10.0%) cases and negative in 29 (58.0%) cases (Table V).

Table V: Distribution of the study cases by Stromal CD 10 status (n=50)

Stromal CD 10 status	Frequency (n)	Percentage (%)
Strong positive	16	32.0
Weak positive	5	10.0
Negative	29	58.0

Association between age with stromal CD 10
Out of total 16 strong positive CD10, 9 (56.3%) cases were grade II and rest 7 (43.8%) cases were grade III (Table VII). The association between tumor grade and stromal CD10 expression were statistically significant.

Table VI: Association between age with stromal CD10 (n=50)

Age (years)	Stromal CD 10			p-value
	Strong positive (n=16)	Weak positive (n=5)	Negative (n=29)	
≤30	1 (6.3)	1 (20.0)	0 (0.0)	0.062
31 - 40	2 (12.5)	1 (20.0)	9 (31.0)	
41 - 50	3 (18.8)	2 (40.0)	11 (37.9)	
51 - 60	8 (50.0)	0 (0.0)	9 (31.0)	
>60	2 (12.5)	1 (20.0)	0 (0.0)	
Mean±SD	50.06 ± 10.70	46.20 ± 15.27	45.55 ± 7.71	0.321[ns]

ns = Non significant, ANOVA test was done to measure the level of significance

Association of tumor size with stromal CD10
Tumor size was between ≤3.0 cm in 8 (50.0%) cases of strong positive group, 3 (60.0%) cases in weak positive group and 15 (51.7%) cases in negative group. The mean tumor size was found 3.94 ± 1.98 cm in strong positive, 3.80 ± 2.49 cm in weak positive and 4.08 ± 2.36 cm in negative. The mean difference was not statistically significant (p>0.05) among three groups (Table VII)

Table VII: Association between tumor size with stromal CD10 (n=50)

Tumor size (cm)	Stromal CD 10			p-value
	Strong positive (n=16)	Weak positive (n=5)	Negative (n=29)	
≤3.0	8 (50.0)	3 (60.0)	15 (51.7)	0.974
3.1-5.0	3 (18.8)	1 (20.0)	7 (24.1)	
>5.0	5 (31.3)	1 (20.0)	7 (24.1)	
Mean±SD	3.94 ± 1.98	3.80 ± 2.49	4.08 ± 2.36	0.959[ns]

ns = Non significant
ANOVA test was done to measure the level of significance

Association of tumor grade with stromal CD10

Out of total 16 strong positive CD, 9 (56.3%) cases were grade II and rest 7 (43.8%) cases were grade III (Table VIII). The association between tumor grade and stromal CD10 expression were statistically significant.

Table VIII: Association between tumor grade with stromal CD10 (n=50)

Tumor grade	Stromal CD 10			p-value
	Strong positive (n=16)	Weak positive (n=5)	Negative (n=29)	
I	0 (0.0)	0 (0.0)	3 (10.3)	0.003[s]
II	9 (56.3)	3 (60.0)	26 (89.7)	
III	7 (43.8)	2 (40.0)	0 (0.0)	

s = significant

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

Discussion

Breast cancer has a major public health implication both in the developed and developing countries as well as is the paramount cause of death in women worldwide with more than one million cases occurring in each year. There have been a lot of evidences in different articles which support tissue microenvironment as having an essential role in controlling cell survival, proliferation, migration, polarization, and differentiation. The prognostic aim of novel stromal marker such as CD10 is less concerning. This study was done to find out the role of CD10 as a prognostic stromal marker in breast carcinoma and to compare CD10 expression with other clinicopathological status in breast cancer. This was a cross-sectional study which was carried out to examine the function of stromal CD 10 as a prognostic stromal marker in breast carcinoma and also to analyze the association between stromal CD10 expression and common clinicopathological parameters (patient age, tumor grade and tumor size). In this study, out of total 50 cases, most (34.0%) of the patients belonged to the age group of 51-60 years followed by 41 -50 years (32.0%), and 31- 40 years (24.0%), >60 years (6.0%) and ≤30 years (4.0%). The mean age was 47.0±9.6 years. In contrast regarding the relation between age with stromal CD10 it was found that the mean age was 50.06 ± 10.70 years which was in strong positive, 46.20 ± 15.27 years which was in weak positive group and 45.55 ± 7.71 years which was included in negative group. The conflict was not accurately significant (p>0.05) among three groups. Similarly observed no statistically significant relationship between stromal CD10 expression and age.[12-15] The tumor size was ≤3.0 cm in 18 (52.0%) patients, 3.1-5.0 cm in 13 (26.0%) patients and >5 cm in 13 (26.0%) patients. Mean tumor size was 4.0±2.21 cm. Tumor size was between ≤3.0 cm in 8 (53.8%) cases of strong positive group, 3 (60.0%) cases in weak positive group and 15 (51.7%) cases in negative group. The mean tumor size was observed 3.94 ± 1.98 cm in strong positive, 3.80 ± 2.49 cm in weak positive and 4.08 ± 2.36 cm in negative. The mean difference was not statistically significant (p>0.05) among three groups. Two studies suggested no association of CD10 with tumor size.[12,16] Some studies[13,14] showed a direct correlation between CD10 expression and tumor size, which differed from the current study.[13,14] In this series it was observed that maximum 38 (76.0%) cases were grade II tumors followed by 09 (18.0%) cases were grade I and 03 (6.0%) cases were grade III tumor. Nine

(56.3%) out of total 16 CD10 strong positive cases, 3 (60.0%) out of five CD10 weak positive and 26 (89.7%) out of 29 CD 10 negative were grade I tumor. Seven (43.8%) were grade II tumor in the strong positive group, 2 (40.0%) in weak positive group and none in negative group. There was a significant ($p<0.05$) association between tumor grade with stromal CD10. In this current study it was observed that all the 16 (100.0%) CD10 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 ($p<0.05$). Similar association was also observed by.[13, 14] In this study progesterone receptor was negative in 34 cases out of total 50 cases. All 16 cases which were strong positive for CD10 were all negative for progesterone receptor. One Single (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 ($p<0.05$). A study [3] showed statistically significant correlation between strong CD10 staining and PR negativity. Other studies found an association between strong stromal CD10 expression with PR negative status.[14,17] In another study, found no statistical significance between stromal CD10 expression and PR status Hilton et al mentioned it was found that progesterone receptor status has not been associated with CD10 expression.[10] Stroma plays a key role in the development, progression, hormonal expression and response to chemotherapy in breast cancer.[10] This has necessitated for the study of stromal markers in breast cancer. CD10, a novel stromal marker plays an important role in normal breast involution and in the development and progression of breast carcinoma. CD10 positive stromal signature also carried prognostic value in breast cancer.[18] All these points to the fact that stroma plays an important role in breast cancer progression and prognostication, and in coming days new indicators such as CD10, TGF-β, SPARC, integrins and laminins are to be used for better prognostication of breast cancer.[19-21] In this study CD10 expression had a strong correlation with well-established prognostic indicators ER/PR negativity and higher tumor grade thus indicating, CD10 can be used as autonomously marker indicating lower prognosis. A very little cohort analyses which established strong CD 10 expression was associated with poor disease free survival rate.[22]

Conclusion

This study was initiated to examine the expression of stromal CD10 in invasive breast carcinoma in mastectomy specimen. Stromal CD10 in invasive breast carcinoma was significantly ($p<0.05$) associated with Tumor grade. ER status, PR receptor status carried a prominent relationship with stromal CD10. On the basis of previous studies, these findings assured that stromal CD10 expression correlated strongly with prognostic indicators ER/PR negativity. Thus indicating, CD10 can be applied as independent marker indicating poor prognosis. Stromal CD10 expression cases have been more likely to show adverse pathological features.

Limitations

Post-operative diagnosis was the main limitations of the present study. Therefore in future, further study may be undertaken by preoperative diagnosis (core needle Biopsy) with large sample size.

Recommendations

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

Reference

1. DeSantis CE, Ma J, Gaudet MM, Newman LA, Miller KD, Goding Sauer A, Jemal A, Siegel RL. Breast cancer statistics, 2019. CA: a cancer journal for clinicians. 2019 Nov; 69(6):438-51.
2. Ferlay JS, Soerjomataram I, Ervik M, Dikshit R, Eser S, Mathers C, Rebelo M, Parkin DM, Forman D, Bray F. GLOBOCAN 2012 v1. 0. Cancer incidence and mortality worldwide: IARC Cancer Base. 2013; 11.
3. Hossain MS, Ferdous S, Karim-Kos HE. Breast cancer in South Asia: a Bangladeshi perspective. Cancer epidemiology. 2014 Oct 1; 38(5):465-70.
4. Ferlay J, Soerjomataram I, Dikshit R, Eser S, Mathers C, Rebelo M, Parkin DM, Forman D, Bray F. Cancer incidence and mortality worldwide: sources, methods and major patterns in GLOBOCAN 2012. International journal of cancer. 2015 Mar 1; 136(5):E359-86.
5. Nishimura R, Osako T, Okumura Y, Hayashi M, Toyozumi Y, Arima N. Ki-67 as a prognostic marker according to breast cancer subtype and a predictor of recurrence time in primary breast cancer. Experimental and therapeutic medicine. 2010 Sep 1; 1(5):747-54.
6. DeSantis CE, Ma J, Gaudet MM, Newman LA, Miller KD, Goding Sauer A, Jemal A, Siegel RL. Breast cancer statistics, 2019. CA: a cancer journal for clinicians. 2019 Nov; 69(6):438-51.
7. Danforth Jr DN. Disparities in breast cancer outcomes between Caucasian and African American women: a model for describing the relationship of biological and nonbiological factors. Breast cancer research. 2013 Jun; 15(3):1-5.
8. DMN DM. Triple assessment of breast–Gold standard in mass screening for breast cancer diagnosis. IOSR J Dent Med Sci. 2013; 7(3): 1-7.
9. Iqbal BM, Buch A. Hormone receptor (ER, PR, HER2/neu) status and proliferation index marker (Ki-67) in breast cancers: Their onco-pathological correlation, shortcomings and future trends. Medical Journal of Dr. DY Patil University. 2016 Nov 1; 9(6):674.
10. Hilton HN, Santucci N, Silvestri A, Kantimm S, Huschtscha LI, Graham JD, Clarke CL. Progesterone stimulates progenitor cells in normal human breast and breast cancer cells. Breast cancer research and treatment. 2014 Feb; 143(3):423-33.
11. Makretsov NA, Hayes M, Carter BA, Dabiri S, Gilks CB, Huntsman DG. Stromal CD10 expression in invasive breast carcinoma correlates with poor prognosis, estrogen receptor negativity, and high grade. Modern Pathology. 2007 Jan; 20(1):84-9.
12. Dhande AN, Khandeparkar SG, Joshi AR, Kulkarni MM, Pandya N, Mohanapure N, Aggarwal A, Patil G. Stromal expression of CD10 in breast carcinoma and its correlation with clinicopathological parameters. South Asian Journal of Cancer. 2019 Jan; 8(01):18-21.
13. Taghizadeh-Kermani A, Jafarian AH, Ashabyamin R, Seilanian-Toosi M, Pourali L, Asadi M, Mashhadi L. The stromal overexpression of CD10 in invasive breast cancer and its association with clincophathologic factors. Iranian journal of cancer prevention. 2014; 7(1):17.
14. Mohammadizadeh F, Salavati M. CD10 expression in stromal component of invasive breast carcinoma: A potential prognostic determinant. Journal of Research in Medical Sciences. 2012 Mar 1; 17:194-199.
15. Witkiewicz AK, Freydin B, Chervoneva I, Potoczek M, Rizzo W, Rui H, Brody JR, Schwartz GF, Lisanti MP. Stromal CD10 and SPARC expression in ductal

carcinoma in situ (DCIS) patients predicts disease recurrence. Cancer biology & therapy. 2010 Aug 15; 10(4):391-6.

16. Jana SH, Jha BM, Patel C, Jana D, Agarwal A. CD10-a new prognostic stromal marker in breast carcinoma, its utility, limitations and role in breast cancer pathogenesis. Indian Journal of Pathology and Microbiology. 2014 Oct 1; 57(4):530.
17. Puri V, Jain M, Thomas S. Stromal expression of CD10 in invasive breast carcinoma and its correlation with ER, PR, HER2-neu, and Ki67. International journal of breast cancer. 2011; Article ID 437957, page 4.
18. Cabioglu N, Summy J, Miller C, Parikh NU, Sahin AA, Tuzlali S, Pumiglia K, Gallick GE, Price JE. CXCL-12/stromal cell–derived factor-1α transactivates HER2-neu in breast cancer cells by a novel pathway involving Src kinase activation. Cancer research. 2005 Aug 1; 65(15):6493-7.
19. Desmedt C, Majjaj S, Kheddoumi N, Singhal SK, Haibe-Kains B, El Ouriaghli F, Chaboteaux C, Michiels S, Lallemand F, Journe F, Duvillier H. Characterization and clinical evaluation of CD10+ stroma cells in the breast cancer microenvironment. Clinical cancer research. 2012 Feb 15; 18(4):1004-14.
20. Dhande AN, Khandeparkar SG, Joshi AR, Kulkarni MM, Pandya N, Mohanapure N, Aggarwal A, Patil G. Stromal expression of CD10 in breast carcinoma and its correlation with clinicopathological parameters. South Asian Journal of Cancer. 2019 Jan; 8(01):18-21.
21. Ulaganathan S. An Analysis of Correlation of Stromal CD10 Expression in Carcinoma Breast NOS Type with ER, PR and HER2/Neu. 2018 Aug; 17 (8):52-59
22. Jemal A, Bray F, Center MM et al. Global cancer statistics. 2011; 61: 69-90. Kamal Uddin AFM, Khan ZJ, Islam J et al. Cancer care scenario in Bangladesh. 2013 Apr-june; 2 (2):102-104