Original Article

DOI: 10.7860/JCDR/2022/55414.16241

Pathology Section

Clinicopathological Study of Breast Lesions with Special Reference to the Role of CD34 Immunostaining in Diagnosis- A Cross-sectional Study from a Tertiary Care Hospital of West Bengal, India

AMBIKA PRASAD CHAKRABORTY[1], DEBASIS MUKHOPADHYAY[2], ARNAB MANDAL[3], APARAJITA SAMADDAR[4], SWAPAN PATHAK[5]

ABSTRACT

Introduction: Breast carcinoma is the most common cancer among women and leading cause of mortality. Prognosis of breast carcinoma primarily depends on early detection. The changes in molecular and antigenic expression in stromal micro-environment surrounding the tumour cells was noted earlier in tumours of skin and gastrointestinal tract which showed loss of Cluster of Differentiation 34 (CD34) expression in stromal cell of malignant tumours.

Aim: To evaluate the intensity of CD34 staining in benign, borderline and malignant lesions of breast and to find out whether it can help to differentiate benign and malignant lesions from borderline and in-situ carcinomas.

Materials and Methods: This was a cross-sectional prospective study done over a period of 18 months in a tertiary care hospital of Bankura, West Bengal, India. Total 78 specimens of breast lesions obtained by lumpectomy and Modified Radical Mastectomy (MRM) were first evaluated on routine histology and classified as benign, borderline or malignant accordingly. Subsequent immunohistochemical staining was performed for CD34 and intensity of expression in stromal cells was graded from 0 to 3+. Then comparison of CD34 expression in different lesions was done and level of significance was assessed by using Student's t-test.

Results: Out of total 78 cases evaluated, 50 (64.1%) were categorised as benign and rest 28 (35.9%) were either premalignant or malignant lesions. Intensity of CD34 expression was found to be significantly higher in benign and in-situ lesions compared to malignant epithelial lesions of breast ($p<0.05$) whereas those between borderline and malignant phyllodes found to be statistically non significant (p-value 0.342).

Conclusion: CD34 immunostaining can help to differentiate benign and in-situ carcinomas from invasive carcinomas, however, its role in differentiating borderline from malignant phyllodes tumour is limited.

Keywords: Breast neoplasms, Immunohistochemisty, Stromal cells

INTRODUCTION

Breast lesions are extremely heterogenous in nature encompassing wide range of benign, borderline and malignant tumours. Breast carcinoma is the second most common cause of cancer death only after lung carcinoma [1]. Though histopathology is considered as gold standard for diagnosis of breast lesions, but immunohistochemical study also plays a crucial role where histological diagnosis is inconclusive especially in differentiating invasive carcinomas from in-situ carcinomas. The growth, differentiation and invasiveness of the breast tumour is tightly regulated by stromal cells including fibroblasts, myoepithelial cells and leukocytes [2]. Normal mammary stroma strongly expresses CD34 in most of the fibrocytes [3]. CD34 is a transmembrane glycoprotein involved in regulation of cell adhesion and signal transduction [4]. It is found in mesenchymal cells of other organs also like prostate, urinary bladder, fallopian tube, thyroid gland, pancreas, colon, uterine cervix and testis [5].

Malignancies arising in aforesaid organs showed loss of CD34 expression and gain of alpha Smooth Muscle Actin (α-SMA) positive myofibroblasts indicating the role of this change in antigenic expression in pathogenesis of tumour progression [4,6]. Similarly in breast carcinoma CD34 positive fibrocytes undergo alteration in morphology and antigenic expression. They acquire plump myofibroblast like appearance and show loss of CD34 expression and acquisition of α-SMA [7]. This is known as myofibroblastic differentiation. It has been suggested that there is an inverse relationship between CD34 expression and myofibroblastic differentiation [8]. Only limited numbers of literatures are available till date to evaluate the diagnostic value of CD34 expression in breast tumours which necessitates further study. With better understanding of changes in stromal micro-environment, this study may help in developing newer target therapy in future. Previous studies did not correlate CD34 expression with clinical staging of breast cancer.

The present study was conducted to evaluate the intensity of CD34 expression in benign, borderline and malignant lesions of breast and also to find out whether it can help to differentiate borderline and in-situ lesions from benign and malignant neoplasms as there is often diagnostic dilemma, experienced on routine histopathology. An attempt was also made to find out the relation between intensity of CD34 staining and clinical stage of malignant breast tumours.

MATERIALS AND METHODS

The present study was a hospital-based prospective cross-sectional study done over a period of 18 months (1st March 2020 to 31st August 2021) in a tertiary care hospital of Bankura, West Bengal, India. The present study was conducted after obtaining ethical clearance from Institutional Ethical Committee (IEC) (No. BSMC/Aca-371 dated 4.2.2020) and informed consents from all the participants. Patient identity was kept confidential.

Inclusion criteria: All the lumpectomy and mastectomy specimens of breast lesions obtained from patients aged ≥18 years presented to the General Surgery Outpatient Department (OPD) with breast lump submitted in properly labeled 10% formalin filled container to the Department of Pathology during the study period with duly filled in histopathology requisition form containing details of patient's age, sex, side of lesion were included in the present study.

Exclusion criteria: The specimens of known inflammatory conditions, infectious diseases or specimens sent without formalin were excluded from the present study.

Sample size calculation: Sample size was calculated by applying the formula $4pq/e^2$; where 'p' is the prevalence. The prevalence of CD34 positivity in stromal cell of high grade DCIS was taken as 12% [9]. So, p=0.12; q=(1-p) and e=allowable error (8% in the present study)=0.08. Thus, the final sample size (n) calculated was 64. To avoid bias, a total of 78 cases of lumpectomy and mastectomy specimens of breast were included in the present study.

Study Procedure

Total 78 cases of lumpectomy and mastectomy specimens of breast were included in the present study. After receiving the sample gross inspection was done and the size and appearance of the tumour, number of lymph nodes etc., were recorded. The sections from the representative areas were submitted for further processing. All the sections were stained by routine Haematoxylin and Eosin (H&E) and examined under light microscope. A histological diagnosis was made including modified Bloom-Richardson histological grading and TNM (tumour (T), nodes (N), and metastases (M) staging was done wherever applicable [10,11]. The epithelial lesions were categorised into benign, premalignant or in-situ and malignant or invasive carcinomas. Phyllodes tumours were categorised into benign, borderline and malignant subtype based on histological findings. Immunohistochemical staining for CD34 was done from the preserved paraffin block using the kit, hermo scientific CD34 (Clone QBEnd/10). Each section was examined and the number of duct/lobular units was identified. The grading of CD34 expression was determined for each duct/lobular unit separately. The sections stained for CD34 were evaluated at high power (x400). Grading was done from 0 to 3+, where:

0: Upto 5% stromal cells immunoreactive

1+: >5 and upto 25% stromal cells immunoreactive

2+: >25 and upto 50% stromal cells immunoreactive

3+: >50% stromal cells immunoreactive

The staining of endothelial cells in blood vessels was taken as internal control.

Grade 0 was interpreted as complete loss of CD34

Grade 1+ was interpreted as reduced expression,

While grade 2+ and 3+ were interpreted as retained expression of CD34 [12]. In the present study, Grade-0 and Grade-1 were considered as CD34 negative; and Grade-2 and Grade-3 were considered as CD34 positive.

STATISTICAL ANALYSIS

Statistical analysis was done using Statistical Package for the Social Sciences (SPSS) software version 13.0 and Student's t-test was performed to assess the level of significance.

RESULTS

Total 78 cases were included in the present study. All the participants were female and 62% of them were more than 45 years old. Of these 50 (64.1%) were benign and rest 28 (35.9%) cases were either premalignant in-situ carcinomas or invasive malignant neoplasms. Most common benign lesion was fibroadenoma (34%) and the most common malignant tumour was invasive carcinoma of no special type (39.3%) [Table/Fig-1]. Out of total 28 cases of malignant breast tumours included in the present study, 15 cases (53.6%) presented with regional lymph node metastasis. Among malignant tumours, nine cases were in clinical stage 0 whereas 14 cases were classified as stage II and five cases were categorised as stage III tumour. On histological grading of 11 cases of invasive carcinoma of no special type, 1 case (9.09%) was categorised as grade 1, 6 cases (54.55%) as grade 2 and 4 cases (36.36%) as grade 3 lesions with grade 2 tumour being the most common type [Table/Fig-2]. All the cases were subjected to CD34 staining. [Table/Fig-1] shows the distribution of different breast lesions in the study population and their intensity of CD34 staining in stromal cells. Most of the benign tumour showed Grade 3 positivity in stromal cells. [Table/Fig-3] is showing Grade 3+ staining in a case of fibroadenoma. However, out of two cases of fibroadenoma with focal Atypical Ductal Hyperplasia (ADH) showed reduced staining intensity for CD34 in the area of ADH as represented by Grade 1+ and Grade 2+ staining pattern. Among the three cases of ADH, 1 case was negative (Grade1+) and 2 cases were positive (Grade 2+). Cases of apocrine metaplasia also

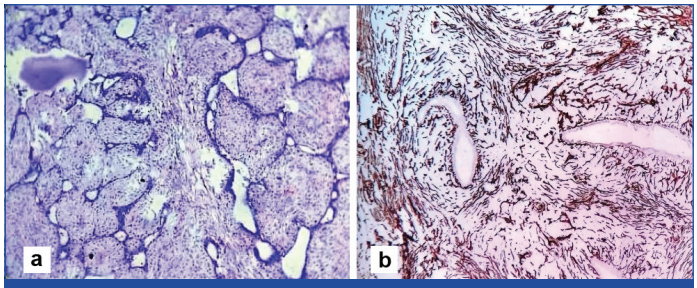
Histological sub-types		No. of cases n (%)	CD34 staining					
			Negative		Positive			
			Grade 0	Grade 1+	Grade 2+	Grade 3+	Total No. of positive cases	% of positivity
Benign lesions	Fibrocystic disease of breast	10 (20)	0	0	0	10	10	100
	Fibroadenoma	17 (34)	0	0	0	17	17	100
	Fibroadenoma with Fibrocystic disease of breast	5 (10)	0	0	0	5	5	100
	Benign phyllodes tumour	4 (8)	0	0	0	4	4	100
	Apocrine metaplasia	3 (6)	0	1	1	1	2	66.67
	Fibroadenoma with ADH	2 (4)	0	1	1	0	1	50
	ADH	3 (6)	0	1	2	0	2	66.67
	Borderline phyllodes tumour	6 (12)	1	2	3	0	3	50
Premalignant and malignant lesions	DCIS	6 (21.4)	4	1	1	0	1	16.67
	LCIS	3 (10.7)	1	1	1	0	1	33.33
	Invasive carcinoma, NOS	11 (39.3)	11	0	0	0	0	0
	Lobular carcinoma	3 (10.7)	2	1	0	0	0	0
	Tubular carcinoma	1 (3.6)	1	0	0	0	0	0
	Medullary carcinoma	1 (3.6)	1	0	0	0	0	0
	Malignant phyllodes tumour	3 (10.7)	2	0	1	0	1	33.33

[Table/Fig-1]: CD34 immunostaining pattern in different breast lesions.

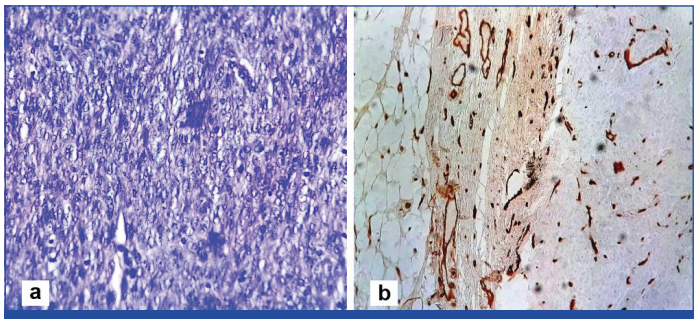
showed reduced expression of CD34. Cases of Ductal Carcinoma In-Situ (DCIS) showed higher frequency of loss of CD34 expression compared to Lobular Carcinoma In-Situ (LCIS) (83.33% vs 66.67%). Complete loss of CD34 expression was found in most of the invasive malignant neoplasms except a single case of malignant phyllodes tumour showed retention of CD34 staining (Grade 2+). [Table/Fig-4] is showing complete loss of CD34 staining in a case of malignant phyllodes tumour.

Parameters assessed		Total no. of cases	No. of cases with loss of CD34 expression	(%)	p-value
Regional lymph node metastasis	Present	15	15	100	Significant (p<0.05)
	Absent	13	10	76.92	
Histological grade	Grade 1	1	1	100	Non significant
	Grade 2	6	6	100	
	Grade 3	4	4	100	

[Table/Fig-2]: CD34 expression pattern in malignant breast tumours in relation to regional lymph node status and histological grade of the lesion.

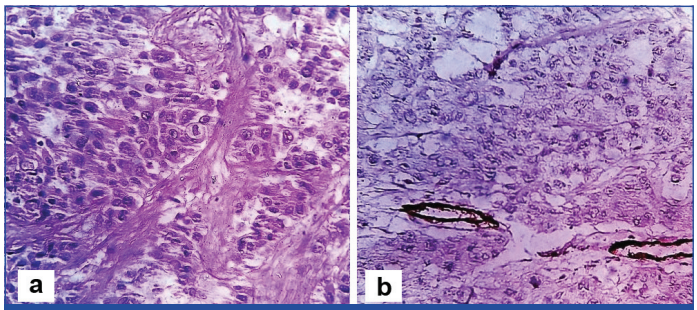


[Table/Fig-3]: Fibroadenoma (a) showing intracanalicular pattern on histology (H&E, x100); (b) Showing Grade 3+ staining in stromal cells (CD34, x100).



[Table/Fig-4]: Malignant phyllodes tumour (a) showing pleomorphic spindle cells on histology (H&E, x400); (b) Showing complete loss of CD34 expression in stromal cells (CD34, x100).

The difference in CD34 expression between benign and both in-situ and invasive malignant tumours were found to be statistically extremely significant (p<0.001) whereas those between borderline and malignant phyllodes found to be statistically non-significant (p-value 0.342). Among tumours of epithelial origin, in-situ carcinomas show statistically significant (p-value=0.0146) retention of CD34 expression in stromal cells compared to invasive carcinomas. As all the cases of invasive carcinoma of no special type showed complete loss of CD34 expression in stromal cells [Table/Fig-5] (Grade 0) irrespective of their histological grade, it can be concluded that CD34 expression cannot help to differentiate breast carcinomas of different histological grade. No statistically significant difference in CD34 expression was noted among tumours of different clinical stages (p-value >0.05). However, malignant tumours without metastasis in regional lymph node showed statistically significant (p<0.05) higher grade of CD34 expression compared to those with lymph node metastasis.



[Table/Fig-5]: Invasive carcinoma of breast, no special type: (a) Showing sheets of atypical ductal cells (H&E, x400); (b) Showing complete loss of CD34 staining in stromal cells (CD34, x400).

DISCUSSION

Breast cancer is the most common cancer among women. Life time risk of breast carcinoma is one in every 10 women [13]. Prognosis of breast cancer largely depends on early diagnosis. Better understanding of underlying molecular and cellular basis of tumour progression can help in early diagnosis and also to develop target therapy in breast carcinomas. The role of stromal microenvironment in tumour progression has long been discussed in relation to various tumours. The stroma surrounding the tumour greatly differs from normal stroma with alteration in protein synthesis and it regulates the proliferation of epithelial component of the tumour [14-16]. Progression of in-situ to invasive carcinoma is an important yet poorly understood area of tumour progression. So differentiating DCIS and LCIS from benign and invasive carcinoma is critically important as earlier diagnosis is the most important step in the management of breast carcinoma. Though histology is considered as the gold standard for diagnosis of breast tumour, but immunohistochemical study has proven to play indispensible role when there is a diagnostic dilemma as often experienced by histopathologists. CD34 is a transmembrane glycoprotein expressed by many cells in our body like haematopoietic stem cells, endothelial cells and messenchymal cells of various organs including stromalfibrocytes of breast. They play a major role in cell adhesion, signal transduction and matrix substance production. It has also been claimed that CD34 molecule may also play role in host response to tissue injury [3,17]. Previous studies have shown that loss of CD34 staining in stromal cells may help to distinguish malignant from benign tumours of skin and gastrointestinal tract [18-20]. Recent studies have also proposed the feasibility of targeting tumour stroma including fibroblasts derived factors for treatment of cancer [3]. The present study was conducted to assess the role of CD34 in differentiating benign lesions from borderline and malignant tumours of breast. Most of the tumours evaluated in present study were benign comprising 64.1% of all tumours with fibroadenoma being the most common type (34%). Malignant and premalihnant tumours constituted 35.9% of the cases with invasive carcinoma of no special type as the major tumour type (39.3%). Distribution of breast tumours found in this study was in concordance with the previous study done by Cimpean AM et al., [2]. In the present study, most of the benign lesions of breast showed Grade 3+ staining for CD34 in stromal cells except three cases of apocrine metaplasia, three cases of ADH and in the focal area of ADH in two cases of fibroadenoma showed Grade 1+ to Grade 2+ staining with positivity rate being 66.67% each for first two categories and 50% for the last one respectively. The finding of the present study was comparable to the previous study by Khan AA et al., [12]. They also observed Grade 2+ to Grade 3+ staining pattern of CD34 in 96% of the cases of ADH [12]. Chauhan H et al., demonstrated diffuse loss of CD34 expression in 50% cases of ADH which is similar to this study [9]. Cimpean AM et al., found retention of CD34 positivity in 100% cases of fibroadenoma with ADH with decreased intensity in their study [2]. Reduced expression of CD34 in the areas of ADH may be indicative of the premalignant nature of the lesion. Similar to this study Khan AA et al., also found reduced intensity of CD34 staining in cases of apocrine metaplasia as represented by Grade 2+ to Grade 3+ staining in only 53.33% cases compared to 66.67% cases in

the present study [12]. The present study depicted statistically significant (p<0.001) loss of CD34 expression in stromal cells of in-situ carcinomas compared to benign lesions indicating their premalignant behaviour. In the present study, out of six cases of DCIS and three cases of LCIS, only two cases including one case from each sub-type showed retention of CD34 expression (Grade 2+). So in the present study, 33.33% cases of LCIS showed CD34 expression. This finding was comparable with Cimpean AM et al., and Chauhan H et al.,they also found retention of CD34 expression in all cases of LCIS [2,9]. Khan AA et al., found CD34 expression in 88.5% of LCIS cases [12]. It has been observed that 20-30% cases of LCIS develop invasive carcinoma subsequently [21]. This indicates the premalignant nature of LCIS which is corroborative with the loss of CD34 staining in a substantial population of cases in the present study. The relation between pattern of CD34 expression and DCIS was found to be variable in different studies. [Table/Fig-6] demonstrating the comparison of CD34 expression pattern in DCIS as found by present and previous studies [2,8,9,12]. So, it was found that CD34 expression in DCIS found in current study was comparable to the pattern observed in high grade DCIS particularly in previous studies. Irrespective of the histological sub-type, all categories of invasive carcinomas including invasive carcinoma of no special type, lobular carcinomas, tubular carcinoma, medullary carcinoma showed complete loss of CD34 staining in stromal cells. This finding was in concordance with previous studies [4,5,8,17,20]. The present study and Cimpean AM et al., found less marked loss of stromal CD34 expression in invasive lobular carcinoma compared to Invasive Ductal Carcinoma (IDC) [2]. In contrast Kuroda N et al., found similar frequency of CD34 loss in both IDC and ILC [22]. The intensity of CD34 expression was found to be significantly higher in-situ carcinomas compared to invasive carcinomas with p-value <0.05. It was concluded that CD34 staining can be utilised to differentiate in-situ from invasive carcinomas when histology alone is inconclusive. In the present study, stromal expression of CD34 was found among 100%, 50% and 33.33% of benign, borderline and malignant phyllodes tumour respectively. Cimpean AM et al., also found loss of CD34 expression in malignant phyllodes tumour [2]. There was a statistically significant difference in CD34 expression among benign and borderline phyllodes tumour, however the difference was not statistically significant between borderline and malignant phyllodes tumour. So, it was concluded that though CD34 staining can be used to differentiate benign phyllodes from borderline or malignant tumours, but it cannot be utilised to differentiate borderline from malignant phyllodes tumour. Overall, the difference in CD34 expression among benign and malignant tumours of breast was found to be highly statistically significant (p<0.001) which was similar to the observation noted by Khan AA et al., [12]. Statistically significant higher intensity of CD34 staining was also noted in carcinomas without regional lymph node metastasis compared to those with lymph node metastasis (p-value <0.05). However, no significant association was found with the histological grade and clinical stage of the tumour.

To summarise, statistically significant (p<0.05) difference in extent of CD34 staining was noted among benign, in-situ and invasive malignant epithelial tumours of breast. So, it can be utilised for differentiating benign from in-situ and in-situ from invasive malignant tumours, especially when there is a diagnostic dilemma on routine histology. However, there was no statistically significant difference in intensity of CD34 expression among borderline and malignant phyllodes tumour in the present study.

Study done by	Publication year	Lesion category	Retention of CD34 expression (%)
Present study	2022	DCIS	16.67
Khan AA et al., [12]	2017	DCIS-Low grade	92
		DCIS-High grade	13
Cimpean AM et al., [2]	2005	DCIS	0
Chauhan H et al., [9]	2003	DCIS-Low grade	60
		DCIS-Intermediate grade	62
		DCIS-High grade	22
Barth PJ et al., [8]	2002	DCIS	0

[Table/Fig-6]: Comparison of CD34 expression pattern in DCIS between present and previous studies [2,8,9,12].

Limitation(s)

Acquisition of α-SMA expression in stromal cells of malignant tumours of breast which is indicative of myofibroblastic differentiation of stromal fibrocytes could not be evaluated in the present study.

CONCLUSION(S)

It was concluded that, CD34 can play crucial role in differentiating benign from in-situ and in-situ from invasive malignant epithelial tumours of breast. It can also be used to differentiate benign from malignant phyllodes tumour of breast, but its role in differentiating borderline from malignant phyllodes tumour is limited.

REFERENCES

[1] Khadem R, Mahdi FC, Al-Mosawi K, AL-Janabi AA. The role of estrogen in breast cancer. Biomed Biotechnol Res J. 2020;4:293-96.
[2] Cimpean AM, Raica M, Narita D. Diagnostic significance of the immunoexpression of CD34 and smooth muscle cell actin in benign and malignant tumors of the breast. Romanian Journal of Morphology and Embryology. 2005;46(2):123-29.
[3] Chesney J, Bacher M, Bender A, Bucala R. The peripheral blood fibrocyte is a potent antigen presenting cell capable of priming naïve T cells in situ. Proc Natl Acad Sci U S A. 1997;94:6307-12.
[4] Van de Rijn M, Rouse RV. CD34 A review. ApplImmunohistochem. 1994;2:71-80.
[5] Yamazaki K, Eyden BP. Ultrastructural and immunohistochemical observations on intralobular fibroblasts of human breast with observations on the CD34 antigen. J Submicrosc Cytol Pathol. 1995;27:309-23.
[6] Kuroda N, Nakayama H, Miyazaki E. Distribution and role of CD34-positive stromal cells and myofibroblasts in human normal testicular stroma, Histol Histopathol. 2004;19(3):743-51.
[7] Wessel C, Westhoff CC, Nowak K, Moll I, Barth PJ. CD34 (+) fibrocytes in melanocytic nevi and malignant melanomas of the skin. Virchows Arch. 2008;453:485-89.
[8] Barth PJ, Ebrahimsade S, Ramaswamy A, Moll R. CD34+ fibrocytes in invasive ductal carcinoma, ductal carcinoma in situ, and benign breast lesions. Virchows Arch. 2002;440:298-303.
[9] Chauhan H, Abraham A, Phillips JRA, Pringle JH, Walker RA, Jones JL. There is more than one kind of myofibroblast: Analysis of CD34 expression in benign, in situ, and invasive breast lesions. J Clin Pathol. 2003;56(4):271-76.
[10] Bansal C, Singh US, Misra S, Sharma KL, Tiwari V, Srivastava AN. Comparative evaluation of the modified Scarff-Bloom-Richardson grading system on breast carcinoma aspirates and histopathology. Cytojournal. 2012;9:4. Doi: 10.4103/1742-6413.92550. Epub 2012 Jan 31. PMID: 22363393; PMCID: PMC3280007.
[11] Singletary SE, Connolly JL. Breast cancer staging: Working with the sixth edition of the AJCC Cancer Staging Manual. CA Cancer J Clin. 2006;56(1):37-47.
[12] Khan AA, Alam K, Harris H. A clinicopathological study of CD34 antigen expression in benign and malignant breast lesions. J Clin Exp Pathol. 2017;7:01-09.
[13] Vultaggio V, Mansi L, Fischer U, Baum F, Luftner-Nagel S. Breast cancer: Diagnostic imaging and therapeutic guidance. Eur J Nucl Med Mol Imaging. 2018;45:24-84.
[14] Bhowmik NA, Neilson EG, Moses HL, Stromal fibroblasts in cancer initiation and progression. Nature. 2004;432(7015):332-37.
[15] Kurose K, Hoshaw-Woodard S, Adeyinka A. Genetic model of multi-step breast carcinogenesis involving the epithelium and stroma: Clues to tumor-microenvironment interactions. Hum Mol Genet. 2001;10:1907-13.
[16] Silberstein GB. Tumor-stromal interactions: Role of stroma in mammary development, Breast Cancer Res. 2001;3:218-23.
[17] Moore T, Lee AHS. Expression of CD34 and BCL-2 in phyllodes tumors, fibroadenomas and spindle cell lesions of the breast. Histopathol. 2001;38:62-76.
[18] Swanson PE, Fitzpatrick MM, Ritter JH. Immunohistologic differential diagnosis of basal cell carcinoma, squamous cell carcinoma, and trichoepithelioma in small cutaneous biopsy specimens. J Cutan Pathol. 1998;25:153-59.
[19] Nakayama H, Enzan H, Miyazaki E, Kuroda N, Naruse K, Differential expression of CD34 in normal colorectal tissue, peritumoral inflammatory tissue, and tumorstroma. J Clin Pathol. 2000;53:626-29.

[20] Nakayama H, Enzan H, Miyazaki E. CD34 positive stromal cells in gastric adenocarcinomas. J Clin Pathol. 2001;54:846-48.
[21] Rosai J. Rosai and Ackerman's Surgical Pathology, 10th ed. Elsevier Inc, 2011; pp. 1665.
[22] Kuroda N, Jin YL, Hamauzu T, Toi M, Miyazaki E, Hiroi M, et al. Consistent lack of CD34-positive stromal cells in the stroma of malignant breast lesions. Histol Histopathol. 2005;20:707-12.

PARTICULARS OF CONTRIBUTORS:
1. Postgraduate Trainee, Department of Pathology, Bankura Sammilani Medical College, Bankura, West Bengal, India.
2. Associate Professor, Department of Pathology, Bankura Sammilani Medical College, Bankura, West Bengal, India.
3. Associate Professor, Department of General Surgery, Bankura Sammilani Medical College, Bankura, West Bengal, India.
4. Assistant Professor, Department of Pathology, Medical College and Hospital, Kolkata, West Bengal, India.
5. Professor, Department of Pathology, Bankura Sammilani Medical College, Bankura, West Bengal, India.

NAME, ADDRESS, E-MAIL ID OF THE CORRESPONDING AUTHOR:
Dr. Aparajita Samaddar,
4/3K/297, Ho Chi Minh Sarani, Sakuntala Park, Kolkata-700061, West Bengal, India.
E-mail: aparajita.samaddar@gmail.com

PLAGIARISM CHECKING METHODS: [Jain H et al.]
- Plagiarism X-checker: Feb 10, 2022
- Manual Googling: Mar 04, 2022
- iThenticate Software: Mar 11, 2022 (9%)

ETYMOLOGY: Author Origin

AUTHOR DECLARATION:
- Financial or Other Competing Interests: None
- Was Ethics Committee Approval obtained for this study? Yes
- Was informed consent obtained from the subjects involved in the study? Yes
- For any images presented appropriate consent has been obtained from the subjects. Yes

Date of Submission: **Feb 08, 2022**
Date of Peer Review: **Mar 05, 2022**
Date of Acceptance: **Mar 17, 2022**
Date of Publishing: **Apr 01, 2022**