

**Role of Stromal Expression of CD34 Immunohistochemical Marker in Proliferative Lesions of Breast: a Diagnostic Study at a Tertiary Care Center in Western Rajasthan**

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**Abstract**

**Introduction:** CD34 antigen is a tool important in differentiating between benign and malignant conditions. This study was aimed to determine the stromal expression of CD34 antigen by immunohistochemical method in proliferative lesions of the breast and to evaluate the specificity of loss of CD34 antigen expression in stromal cells for malignant lesions.

**Materials and methods:** 90 cases of proliferative lesions of the breast were studied from January 2022 to December 2022. Paraffin-embedded blocks were retrieved for all the cases and underwent routine processing followed by Hematoxylin and Eosin staining. 54 cases of the benign lesion and 36 cases of the malignant lesion were studied for CD34 immunohistochemical staining, and semi-quantitative assessment was done.

**Results:** Out of total 35 cases having grade-0 expression, majority are Infiltrating Duct Carcinoma (19 cases) followed by Invasive Breast Carcinoma (13 cases, small biopsies).

We had 1 cases of grade-1 expression and it was Complex Fibroadenoma.

Out of 14 cases having grade-2 expression, majority were fibroadenoma (6 cases) followed by 2 cases of tubular adenoma, 1 case each of benign phylloides, borderline phyllodes tumor, fibroadenosis, fibroadenomatoid hyperplasia, fibroadenoma with apocrine changes and fibroadenoma with epithelial hyperplasia.

Out of 40 cases having grade-3 expression, majority were fibroadenomas (29 cases) followed by 3 cases each of fibroadenoma with fibrocystic disease and fibroadenoma with epithelial hyperplasia, 2 case each of fibrocystic disease and fibroadenoma with apocrine changes and 1 case of epithelial hyperplasia.

**Conclusion:** CD34 antigen expression in stromal cells can be used as an adjuvant tool in differentiating between benign and malignant conditions where the morphology is equivocal.

**Keywords:** Breast; Proliferative lesions; Immunohistochemical; Stromal cells

## Introduction

Normal mammary stroma contains a large number of CD34 positive fibroblasts which can be detected at the 10<sup>th</sup> week of gestation and in developmental phase these can be found in the majority of stromal cells [1]. Normal breast shows CD34 positivity in both intralobular and interlobular stroma where the intralobular stroma has strong positivity, and interlobular stroma has weak positivity for CD34. CD34 positive stromal cells can also be present in endothelial cells of vessels. In many studies, the malignant transformation of the tumours shows a reduction of CD34 positive stromal fibroblasts [2, 3]. The importance of stromal interaction with the ductal system is important for normal breast development and tumorigenesis in malignancy [4-6].

During the tumorigenesis, mutation in the premalignant ductal epithelial cells activates the adjacent stroma, and the activated stromal fibroblasts undergo morphological and phenotypical alterations which include loss of CD34 expression and acquire smooth muscle actin when it becomes myofibroblast-like cells which allow the malignant cells to disseminate through the stroma because of the contractile property [7]. Tumor microenvironment exerts a major modulatory effect on epithelial tumors and the stroma makes an important contribution to the process of tumor progression [8]. The stroma around invasive breast tumours is known to differ from normal breast, with alterations in stromal protein synthesis [9]. CD34+ fibrocytes are capable of collagen I and collagen III synthesis, it seems to be likely that this cell type may be involved in the process of stromal remodeling in breast cancer [10].

## 1. Materials and methods

The current study was done in department of Pathology, Dr. S.N. Medical College and associated hospitals, Jodhpur. It is analytical observational study on cases of Proliferative Lesions of Breast from January 2022 to December 2022. Ethical clearance was obtained from the college Ethical Committee. (SNMC/IEC/2022/Plan1649/7.10.22)

Paraffin blocks were retrieved, sections cut at 4 micron and immunostaining was done on automated immunostainer Leica bond max. using CD34 antibody from dako. A brown coloured cytoplasm of stromal fibroblast was taken as positive and normal breast parenchyma and endothelial cells of vessels staining served as internal control. A total of 90 cases taken which included 36 malignant and 54 benign masses.

**Table 1- Grading of CD34 immunostaining [11].**

CD 34 % positivity	Grade
<5% of stromal cells	0

5-25%	1
26-50%	2
>50%	3

**Table 2- Semi-quantitative assessment of CD34 immunostaining [11]**

Grade	Expression of CD 34
Grade 0	Interpretate as complete loss of CD 34
Grade 1	Interpretate as reduce expression of CD 34
Grade 2	Interpretate as retain expression of CD 34
Grade 3	Interpretate as retain expression of CD 34

Positive(grade 2 and grade 3); Negative(grade 0 and grade 1)[12]

**(A) INCLUSION CRITERIA:**

This study were done during the time period from January 2022 to December 2022. All patients who have undergone modified radical mastectomy, Simple Mastectomy with Axillary Clearance, Lumpectomy, small biopsies from the breast lesions.

**(B) EXCLUSION CRITERIA:**

1. Biopsy of specimen of patient on post Radiotherapy and post chemotherapy.
2. Inadequate sample.
3. Insufficient fixation.

**Results**

Table: 3 correlation between CD-34 expression and histopathological diagnosis of tumour.

Histopathological Diagnosis	Grade 0	Grade 1	Grade 2	Grade 3
Benign Phylloides	0	0	1	0
Borderline Phyllodes Tumor	0	0	1	0
Complex Fibroadenoma	0	1	0	0
Epithelial Hyperplasia	0	0	0	1
Fibroadenosis	0	0	1	0
Fibroadeomatoid Hyperplasia	0	0	1	0
Invasive Duct Carcinoma	1	0	0	0
Invasive Lobular Carcinoma	1	0	0	0
Medullary Carcinoma Of Breast	1	0	0	0
Fibrocystic Disease	0	0	0	2
Tubular Adenoma	0	0	2	0
Fibroadenoma With Apocrine Changes	0	0	1	2

Fibroadenoma With Fibrocystic Disease	0	0	0	3
Fibroadenoma With Epithelial Hyperplasia	0	0	1	3
Infiltrating Duct Carcinoma	32	0	0	0
Fibroadenoma	0	0	6	29
Total	35	1	14	40

Out of total 35 cases showing grade-0 expression, 32(91.4%) are Infiltrating Duct Carcinoma .

We have 1 cases of grade-1 expression and that was Complex Fibroadenoma.

Out of 14 cases having grade-2 expression, majority were fibroadenoma (6 cases) followed by 2 cases of tubular adenoma, 1 case each of benign phylloides, borderline phylloides tumor, fibroadenosis, fibroadeomatoid hyperplasia, fibroadenoma with apocrine changes and fibroadenoma with epithelial hyperplasia.

Out of 40 cases having grade-3 expression, majority were fibroadenomas (29 cases) followed by 3 cases each of fibroadenoma with fibrocystic disease and fibroadenoma with epithelial hyperplasia, 2 case each of fibrocystic disease and fibroadenoma with apocrine changes and 1 case of epithelial hyperplasia.

Table: 4 Accuracy of CD-34 expression staining

		CD34 Expression	
		Negative (Grade-0,1)	Positive (Grade-2,3)
Histopathology	Malignant	35	1
	Benign	1	53
Sensitivity		97.22%	
Specificity		98.15%	
Disease Prevalence		40%	
PPV		97.22%	
NPV		98.15%	
Accuracy		97.78%	
P-value		<0.0001	

Here, we calculated that CD34 staining is 97.22% sensitive, 98.15% specific, 97.78% accuracy in differentiating between benign and malignant breast lesions .

**Discussion**

reported that Most of the benign lesions showed a similar pattern of CD34 expression with dense, diffuse staining for CD34 in the stroma (grade 2+ to 3+). The cases of atypical ductal hyperplasia showed grade 2+ to 3+ expression in 96% cases (24/25

cases) with reduced expression in one case only[13]. In comparison demonstrated in their study that 50% of cases of ADH showed diffuse staining for CD34 and 50% had partial loss around a duct[14]. However, in another study showed negative to focal CD34 staining in cases of papilloma, in contrast to other benign lesions[15]. study demonstrated Loss of CD34 was seen in 44% cases of malignant Phyllode`s tumor (4 of 9 TDLUs) as compared to 0% cases of benign Phyllode`s. Papillary carcinoma also showed complete loss of CD34 expression in stroma. This is in contrast to cases of papilloma, which showed grade 1+ to 2+ staining[13]. study showed that presence of stromal positive CD34 fibroblasts has been shown to be associated with benign lesions[12]. In their study CD34 positive fibroblasts were found in normal mammary gland and benign lesions of the breast. CD34-positive stromal cells are present in the normal breast [16]. In our study diffuse CD34 expression was seen in the interlobular as well as intralobular stroma of the normal breast . However, the origin of these stromal cells remains controversial with some researchers advocating that CD34+ fibroblasts are present in stroma since birth [16], while others are of the view that they are derived from blood borne fibrocytes . CD34 fibrocytes also play a role in angiogenesis via bFGF, VEGF, PDGF-a, IL-8, and MMP-9. the stromal reaction induced by carcinomatous lesions leads to acquisition of SMA expression and in turn to stabilization of the lesion (wound contraction) that helps prevent the spread of tissue damage. this may reflect a defense mechanism against “stromal invasion” that induces a phenomenon of stromal healing and stabilization. However, the phenotypic transformation of CD34 fibrocytes into SMA myofibroblasts could also cause the loss of most essential functions (including immunity, cell adhesion, motility, stromal remodeling and angiogenesis inhibition), and in a paradoxical manner promote tumorigenesis, thus facilitating invasion and metastatic dissemination of tumor cells [17].

## Conclusion

Besides being expressed in the wall of blood vessels, there is diffuse expression of CD34 in the normal breast stroma. Its expression is retained in most of the benign breast lesions. The expression of CD34 in stroma of malignant lesions is almost completely lost in all types of malignant neoplasms. Due to the significant differences in CD34 expression between benign and malignant breast lesions, it can potentially be used to differentiate between the two and can be used as an important diagnostic marker. In future, further studies can also be done to establish its role as therapeutic target in cases of breast cancer.

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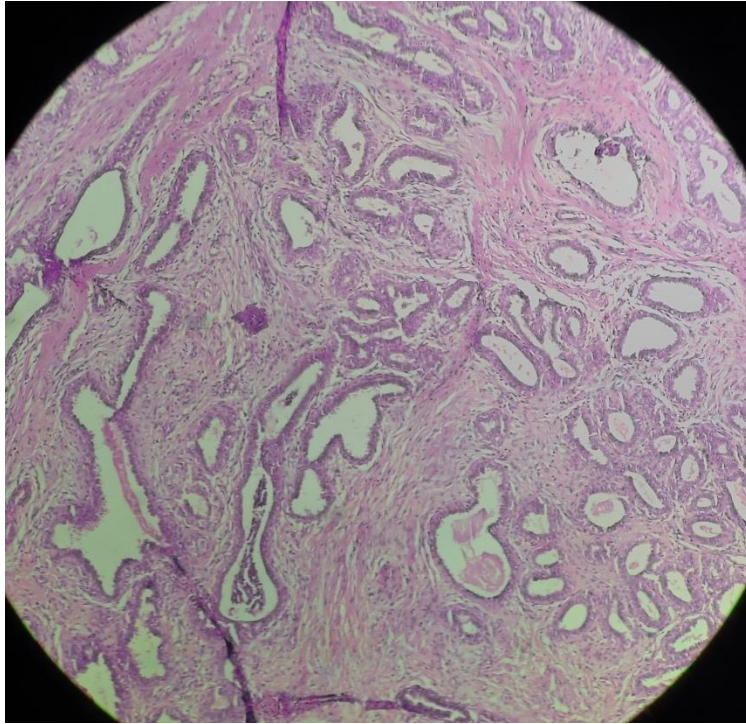
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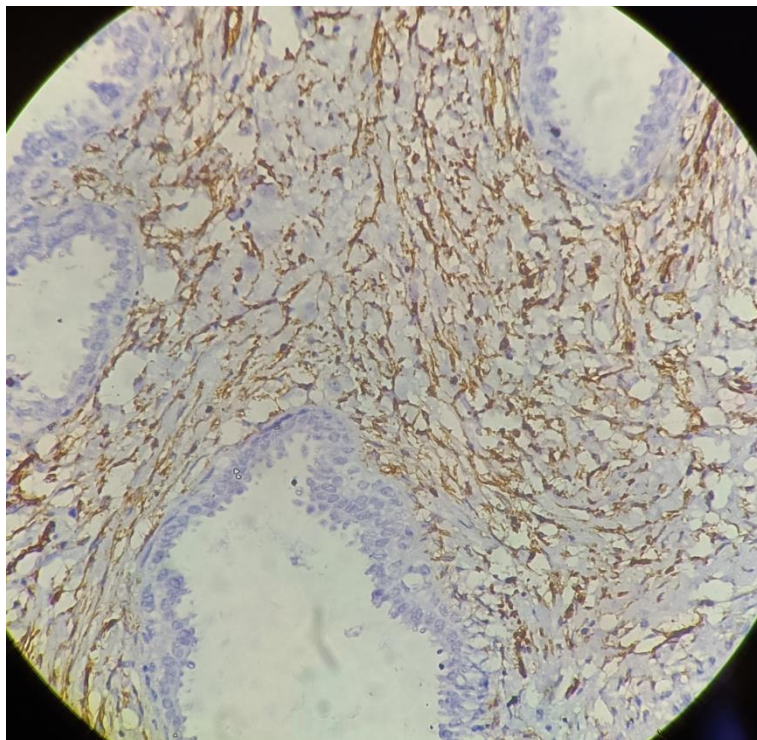
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IMAGES

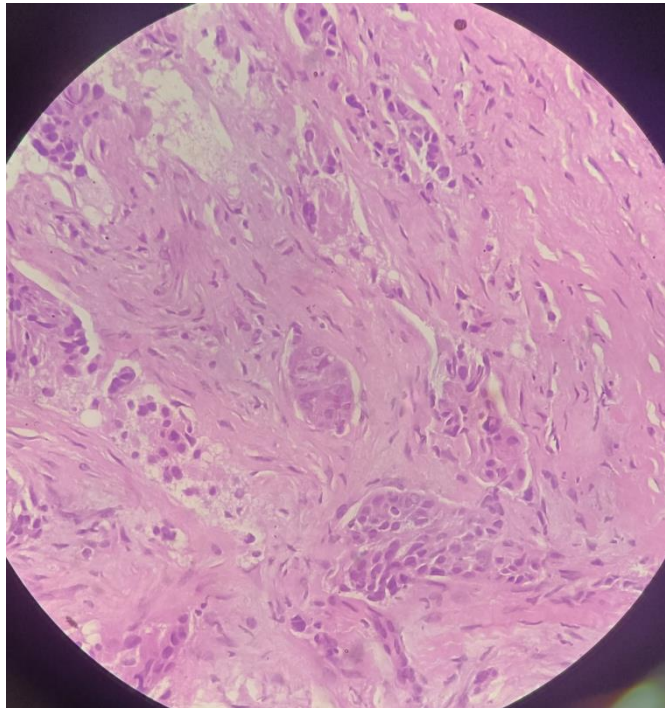


**Figure 1 : H & E picture of Fibroadenoma(x100)**

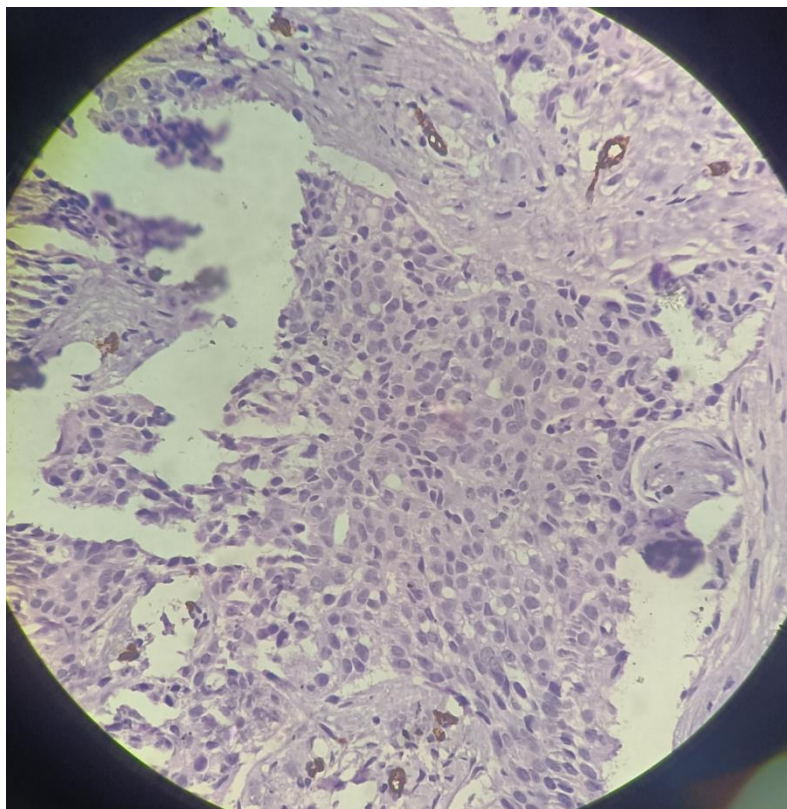


**Figure 2 : Immunohistochemistry of CD34 Expression in Fibroadenoma showing cytoplasmic positivity (x400)**





**Figure 3 : H & E picture of Infiltrative Ductal carcinoma(x100)**



**Figure 4 : Immunohistochemistry of CD34 Expression in Infiltrative Ductal**

**carcinoma showing cytoplasmic negativity / No Staining(x400)**