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Immunohistochemical Evaluation of Antigenic Expression of CD34 in Benign and Malignant Breast Lesions

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Abstract

Background: Immunohistochemistry plays a role in differentiating between benign and malignant lesions of the breast where morphological features are equivocal. The present study was conducted to demonstrate the expression of CD34 antigen using immunohistochemical staining in the stroma of non-neoplastic and neoplastic breast lesions. The aim of the study was to examine the role of CD34 in differentiating between benign and malignant diseases of the breast and to evaluate whether the loss of CD34 was specific for malignant breast lesions.**Material and Methods:**This was an observational study conducted over a period of two years in the Department of Pathology, Government Medical College Patiala. Breast tissue obtained after biopsy and mastectomy procedures from 100 female patients between the ages of 15 and 85 years was used for the study. The IHC sections stained for CD34 were evaluated and grading was done. Statistical test for P value was used to calculate the Significance between Benign andMalignant lesions.**Results:**Out of 53 cases with positive expression of CD34, 47 cases were benign, 1 was malignant and 5 were non-neoplastic. All 47 cases with negative expression of CD34 belonged to malignant type.**Conclusion:**The study showed significant differences between the CD34 expression in benign and malignant breast lesions which can be used to differentiate between the two. Moreover, it can be used as a diagnostic marker to study the progression of lesion. It can also be used as an adjunct with other markers already in use. However, further studies should be undertaken to establish its role as a therapeutic target in breast cancer lesions.

Keywords: Breast, CD34, Immunohistochemistry.

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Introduction

The breast lesions can be divided into non- neoplastic and neoplastic. Neoplastic categories are further of benign and malignant type. In addition to these, metastatic lesions are also seen in the breast.

Fibroadenoma is the most common benign tumor of the breast with incidence of 25%.[1] Breast cancer is the most common female cancer worldwide representing nearly a quarter (25%) of all cancers with an estimated 1.67 million new cancer cases diagnosed in 2012. Taking population growth into consideration, experts predict that there will be about 3.2 million new breast cancer cases per year globally by 2050.[2]

Various diagnostic modalities exist for detecting and classifying breast tumours. Breast lesions can be diagnosed clinico-radiologically, and can be sampled by Fine needle aspiration, core needle biopsy and surgical excision. However, challenges remain in the

ISSN: 0975-3583,0976-2833 VOL13, ISSUE 05, 2022

practice of cytopathology and histopathology and a specific diagnosis can be arrived at with the help of immunohistochemistry.[3] Immunohistochemistry (IHC) is a technique for detecting antigens (such as proteins) in the cells of a tissue segment using antibodies that bind specifically to the antigens in biological tissues.[4]

Malignant lesions are prognostically graded after diagnosing on ER, PR and HER 2neu staining but no specific stain is there to differentiate benign and malignant neoplasms on IHC. CD34 is a highly glycosylated transmembrane protein with a molecular weight of 110 kDa that belongs to the Sialomucin family of cell surface proteins. It also includes Podocalyxin, Thrombomucin, and Endoglycan.[5]

Normal mammary stroma harbor huge numbers of CD34 expressing fibrocytes, which can first be detected during the 10th gestational week. In this developmental phase they make up the majority of stromal cells.[6]

It is also expressed by hematopoietic stem cells, endothelial cells and mesenchymal cells in different tissues, like breast, dermis, thyroid gland, uterine stroma, submandibular gland, intestine and nervous tissue .It can be expressed by embryonic fibroblasts.[7-15]

CD34 is thought to be involved in the modulation of signal transduction.[7] The other known functions of this protein include cellular adhesion, e.g: homing of T lymphocytes in lymph nodes via, L-selectin,[5,9] trafficking of hematopoietic cells, enhancing proliferation and blocking differentiation.[5]

The stroma around invasive breast tumours is known to differ from normal breast, with alterations in stromal protein synthesis,[16] and expression of Matrix Metalloproteinase (MMP).[17,18]

Several studies have shown the loss of CD34 fibrocytes to be a feature of stromal alterations associated with invasive carcinomas of the breast.[19,20,21]

These data indicate a strong negative association between the presence of CD34 positive fibrocytes and the malignancy of ductal system of breast.[22]

The aim of the present study was to demonstrate the expression of CD34 antigen using immunohistochemical staining in the stroma of normal mammary glands, benign and malignant breast lesions and to study the role of CD34 in differentiating between benign and malignant diseases of the breast. The study also evaluated whether the loss of CD34 is specific for malignant breast lesions.

Material and Methods

The present study is an observational study conducted over a period of two years in the Department of Pathology, Government Medical College Patiala. The study was conducted on 100 female patients of all ages with non- neoplastic and neoplastic breast lesions who underwent surgery as a primary modality of treatment. Inadequate tissue sample, male breast lesions and follow up cases were not included in this study.

The study was done after approval from Research and Ethics Committee, Government Medical College, Patiala.

Material

A) Sample Selection- Breast tissue obtained after biopsy and mastectomy procedure was formalin fixed, processing done in Histokinette automatic tissue processor followed by paraffin embedding. Blocks made were used for microtomy. Sections were cut from blocks and stained by-

1) H&E routine stain

2) IHC staining for CD34.

B) Methods- Following methods were used for staining procedures-

1) H & E Method

ISSN: 0975-3583,0976-2833 VOL13, ISSUE 05, 2022

2) Immunohistochemical study method: CD34 staining was done using Rabbit monoclonal antibody (clone EP88, Bio SB, CA 93111, USA). Grading was done based on the intensity and number of cells stained using this criteria-

Steps of IHC- Following four main steps were followed:

1. Fixation
2. Antigen retrieval
3. Blocking
4. Antibody labeling and visualization under microscope

Interpretation of the IHC Scoring,[63]

The IHC sections stained for CD34 were evaluated and grading was done from 0 to 3+ as follows-

Upto 5% stromal cells immunoreactive	0
>5% and upto 25% stromal cells immunoreactive	1+
>25% and upto 50% stromal cells immunoreactive	2+
>50% stromal cells immunoreactive	3+

Grade 0	Interpreted as complete loss of CD34.
Grade 1+	Interpreted as reduced expression of CD34
Grade 2+ and 3+	Interpreted as retained expression of CD34.

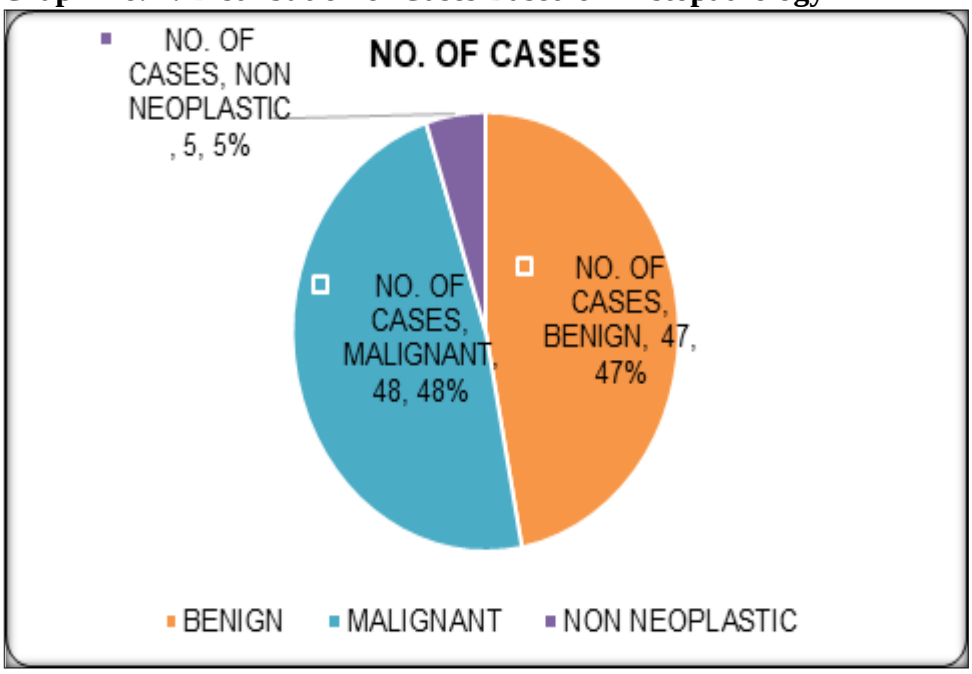
Positive staining was taken as strong intensity of brown in the stromal cells. For comparison, staining in the endothelial cells of blood vessels was seen for internal control.

Statistical Test: P value was used to calculate the Significance between Benign and Malignant lesions.

Results

The range of age distribution varied from less than 20 years to more than 80 years. The youngest patient in our study was of 15 years of age and the oldest patient was of 86 years of age. The mean age of the patients in our study was 41 years.

Graph No. 1: Distribution of Cases Based on Histopathology



ISSN: 0975-3583,0976-2833 VOL13, ISSUE 05, 2022

Out of 100 cases in our study, 95% cases were neoplastic and 5% non-neoplastic. The neoplastic cases comprised of 47% benign and 48% malignant cases.

Table No 1: Distribution of Cases Based on Histopathological Diagnosis and IHC Grading of CD34 (N=100)

Nature	Lesion	No. of Cases	Percentage	Grade 0	Grade 1	Grade 2	Grade 3
Non neoplastic (5cases)	Organizing Breast Abscess	2	2%				
	Fibrocystic Breast Disease	3	3%				
Neoplastic (95 cases)	Benign (47)						
	Fibroadenoma	33	33%			2	31
	Complex Fibroadenoma	2	2%			2	
	Proliferative breast disease without atypia	1	1%				1
	Fibroadenosis	2	2%				2
	Benign PhyllodesTumor	4	4%			2	2
	Borderline PhyllodesTumor	2	2%		1	1	
	UDH	2	2%			1	1
	Intraductal Papilloma	1	1%			1	
	Malignant (48)						
	IDC (NOS)	39	39%	39			
	IDC with medulary features	1	1%	1			
	Invasive Lobular Carcinoma	3	3%	3			
	Mucinous Carcinoma	2	2%	2			
	Micropapillary Carcinoma	1	1%	1			
	DCIS	2	2%	1		1	

Table No 2: IHC Grading of CD34&Histpathology (N=100)

Histopathology	No. of Cases	IHC Grading For CD34			
		Grade 0	Grade 1	Grade 2	Grade 3
Non Neoplastic	5	0	0	0	05
Benign	47	0	01	09	37
Malignant	48	47	0	01	0
Total	100	47	1	10	42

ISSN: 0975-3583,0976-2833 VOL13, ISSUE 05, 2022

The non-neoplastic lesions were 3 fibrocystic breast diseases and 2 organizing breast abscesses. Retained expression of CD34 was seen in 100% of non- neoplastic cases.Out of the 47 benign cases, 19.1% had Grade 2 and 78.7% had Grade 3, 2.1% had Grade 1. Out of 47 benign cases in the study, 46 (97.9%) had retained expression of CD34 (Grade 2 & 3) and only 1 (2.1%) had reduced expression of CD34 (Grade 1). This was consistent with studies done by Khan et al and Elancheran et al.

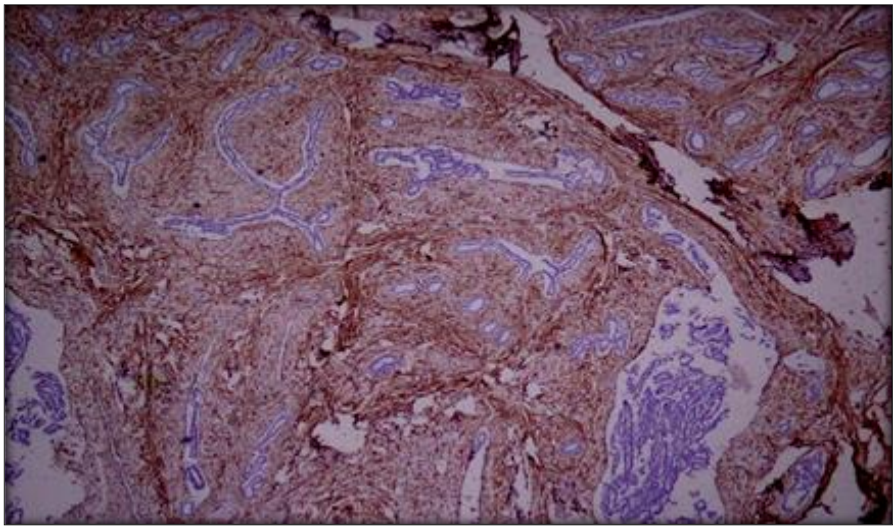


Figure 1:Fibroadenoma Breast; IHC X100 showing retained CD34 expression

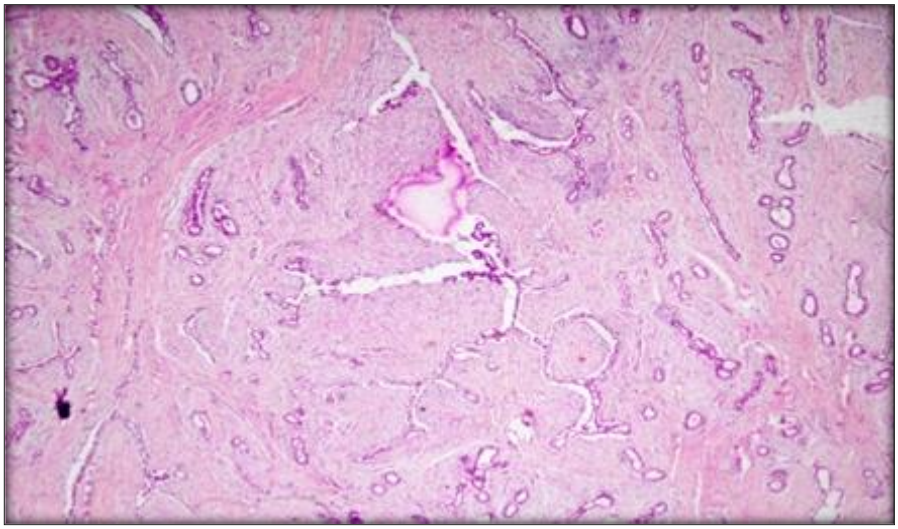


Figure 2:Fibroadenoma Breast (H & E x100)

ISSN: 0975-3583,0976-2833 VOL13, ISSUE 05, 2022

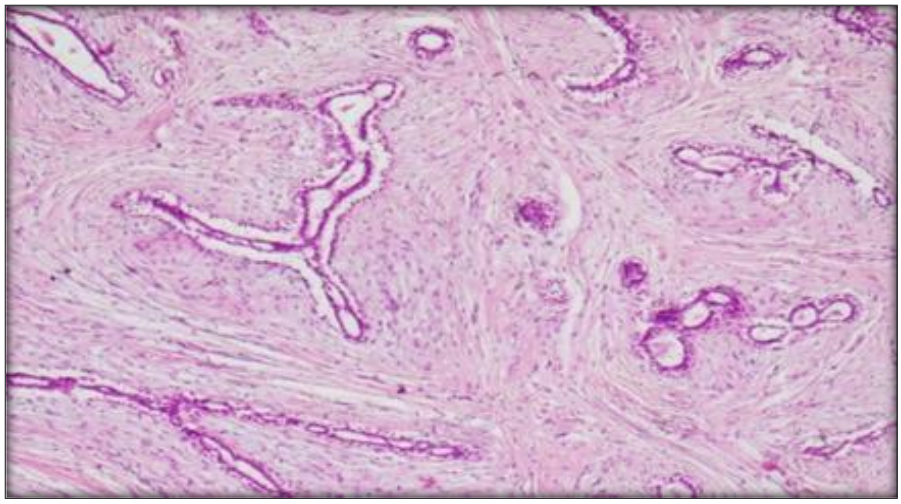


Figure 3:Fibroadenoma Breast, H&E X400

Maximum benign cases were of Fibroadenoma (33). Out of these, 31 were found to have Grade 3 on IHC and 2 had Grade 2. Both the cases of complex fibroadenoma had Grade 2 and both the cases of Fibroadenosis had Grade 3. There was one case each of Proliferative breast disease without atypia and UDH with fibrocystic change which showed Grade 3 on IHC. There was one case each of UDH and Intraductal Papilloma which showed Grade 2. There were 4 cases of Benign PhyllodesTumor, out of which 2 cases showed Grade 2 and 2 cases showed Grade 3. We had 2 cases of Borderline PhyllodesTumor, comprising with 1 case each of Grade 1 and Grade 2.

The malignant lesions were mainly of Intraductal Carcinoma NOS (39 cases). Subtypes of Lobular carcinoma were diagnosed in 3 cases, Mucinous carcinoma in 2 cases and in 1 case each of IDC with medullary features and Micropapillary carcinoma. 2 cases of premalignant lesion seen as DCIS were noted.

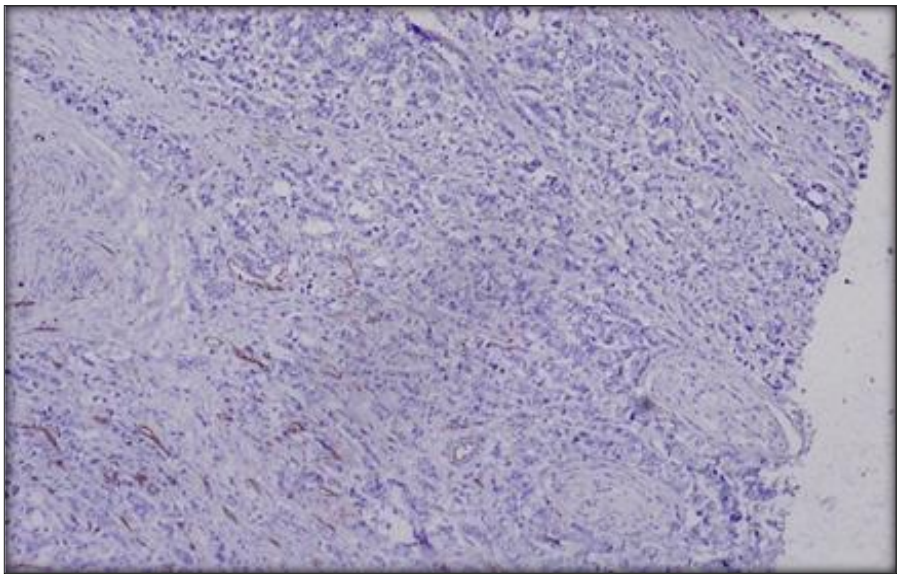


Figure 4: IDC (NOS), IHC X100 showing loss of CD34 expression.

ISSN: 0975-3583,0976-2833 VOL13, ISSUE 05, 2022

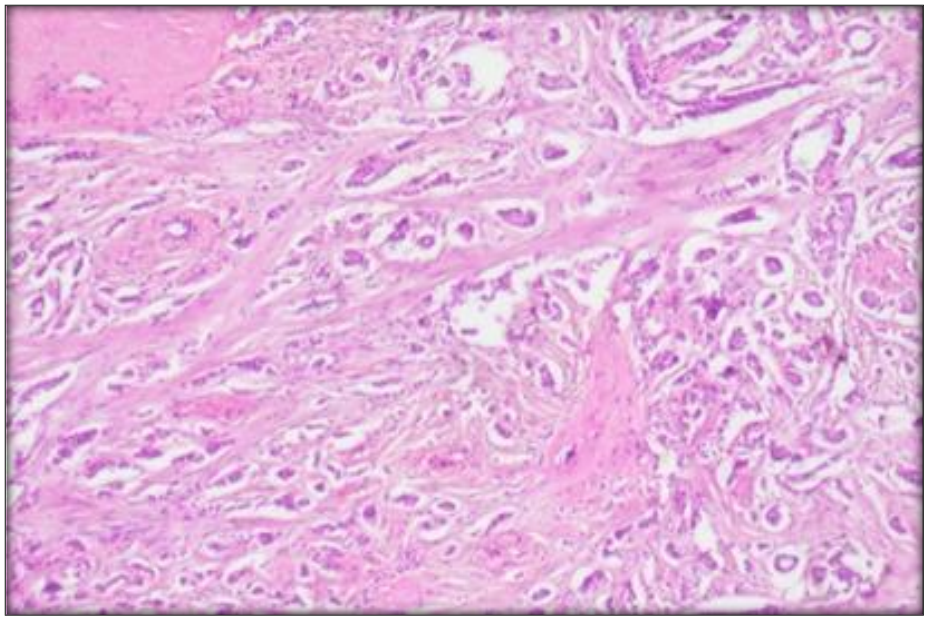


Figure 5: IDC (NOS) breast, (H & E X100)

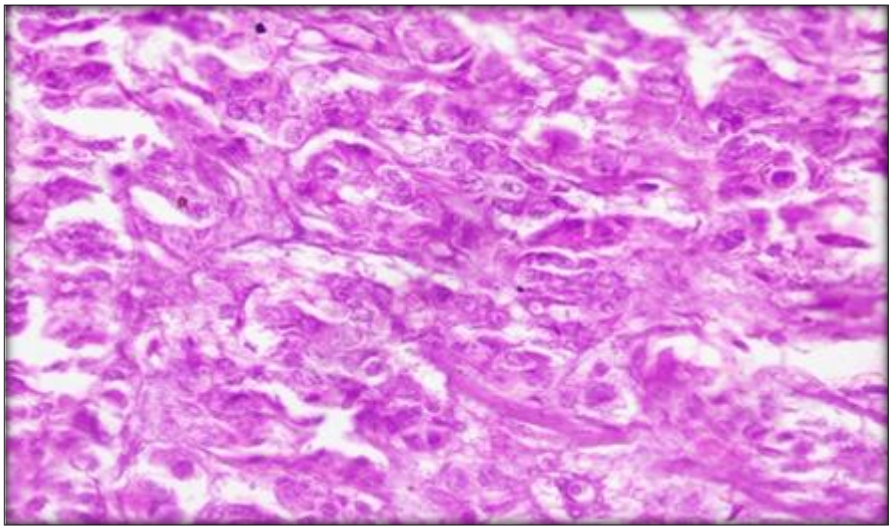


Figure 6: IDC (NOS) Breast, (H & E X400)

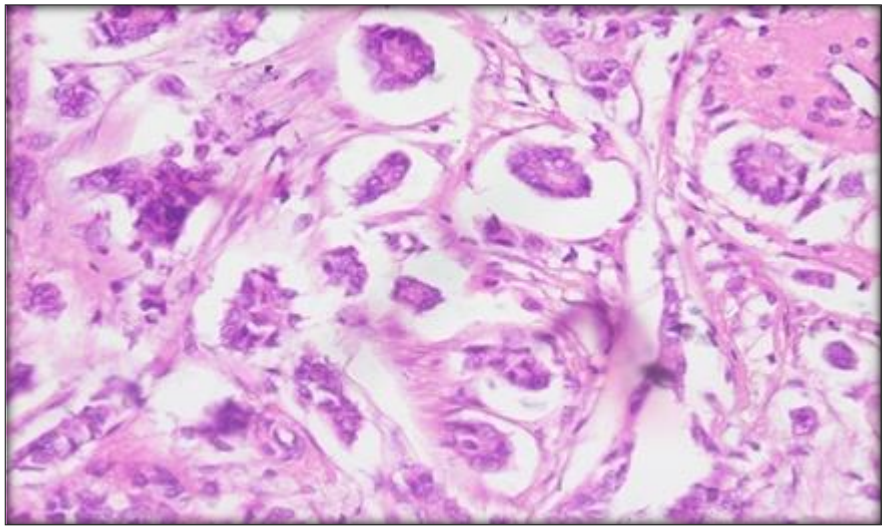


Figure 7: IDC Breast (H & E X400)

ISSN: 0975-3583,0976-2833 VOL13, ISSUE 05, 2022

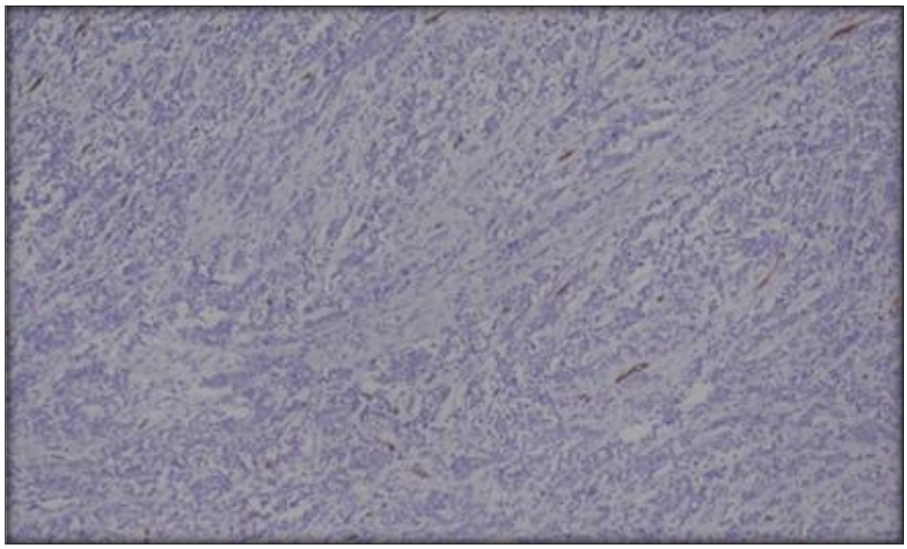


Figure 8: Invasive Lobular Carcinoma (IHC X100) showing loss of CD34 expression

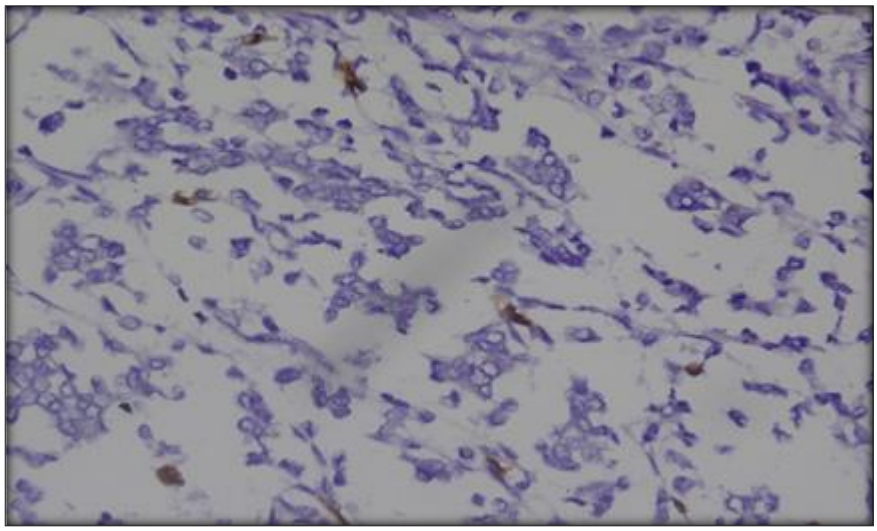


Figure 9: Invasive Lobular Carcinoma (IHC X400) showing loss of CD34 expression

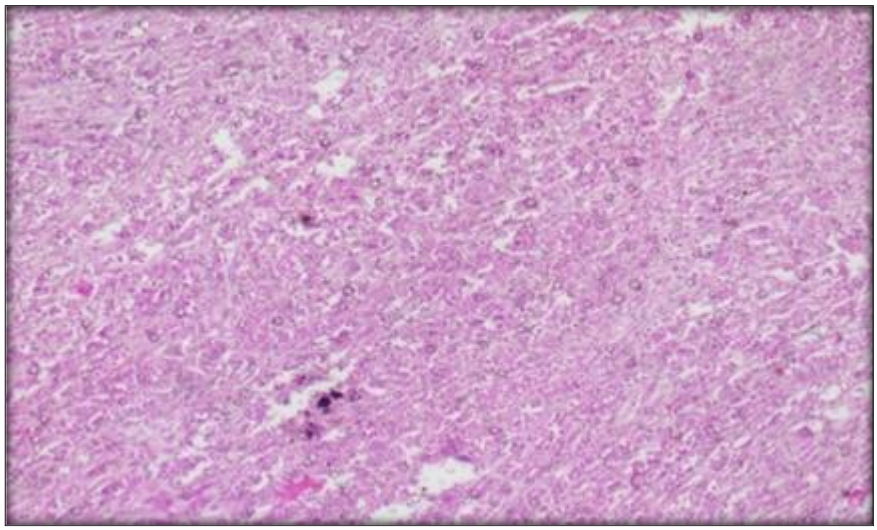


Figure 10: Invasive Lobular Carcinoma (H & E x400).

ISSN: 0975-3583,0976-2833 VOL13, ISSUE 05, 2022

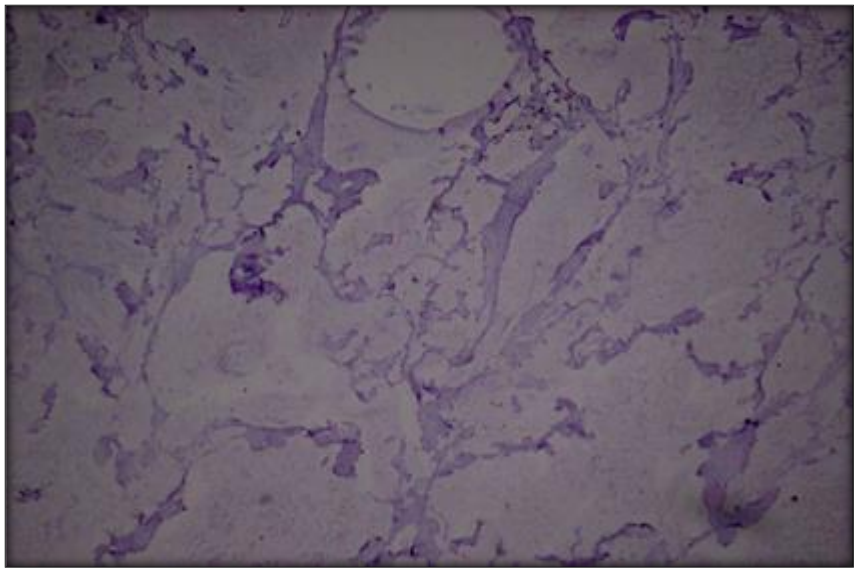


Figure 11: Mucinous Carcinoma (IHC x400) showing loss of CD34 expression.

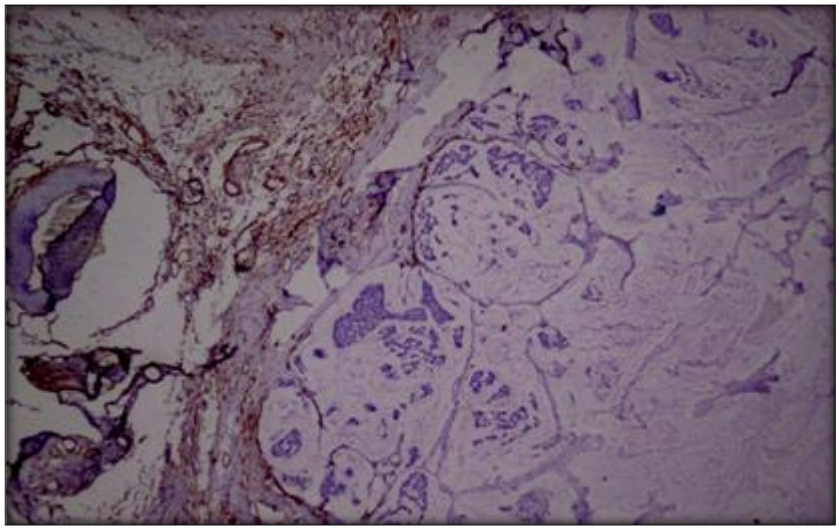


Figure 12: Mucinous Carcinoma, IHC x40 showing loss of CD34 expression in tumor cells floating in the mucin

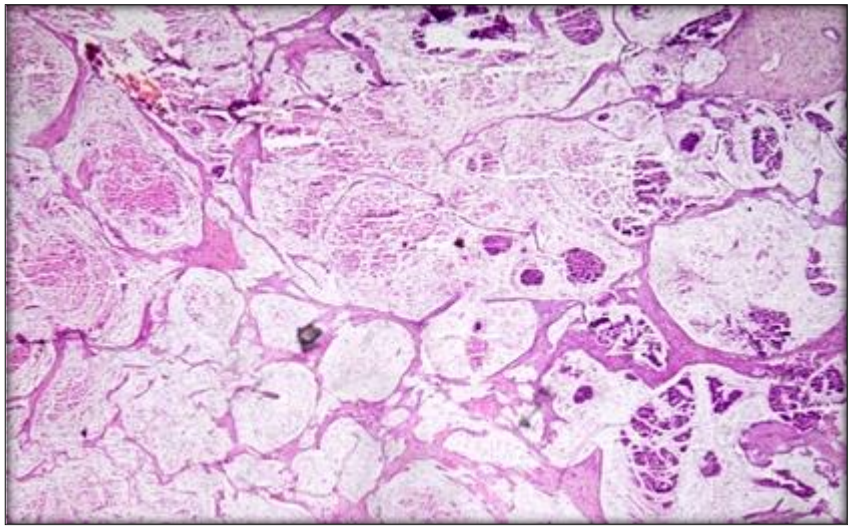


Figure 13: Mucinous carcinoma Breast (H & E x40)

ISSN: 0975-3583,0976-2833 VOL13, ISSUE 05, 2022

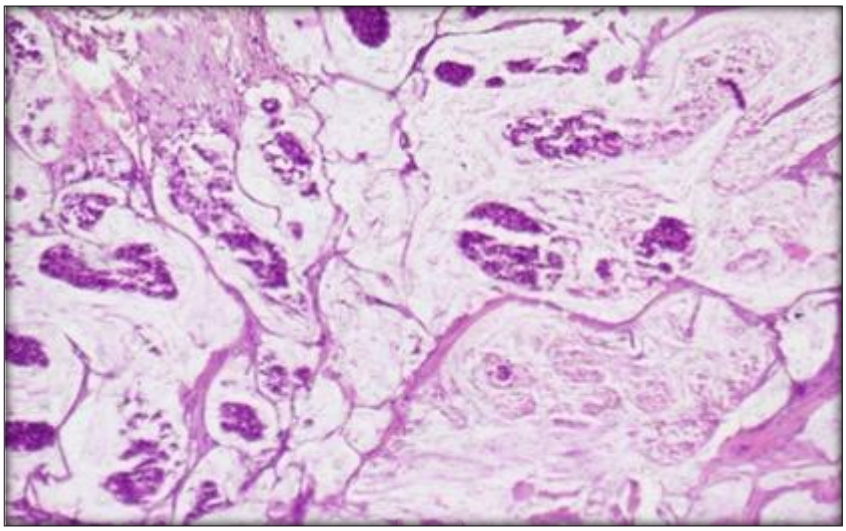


Figure 14: Mucinous carcinoma Breast (H & E x400).

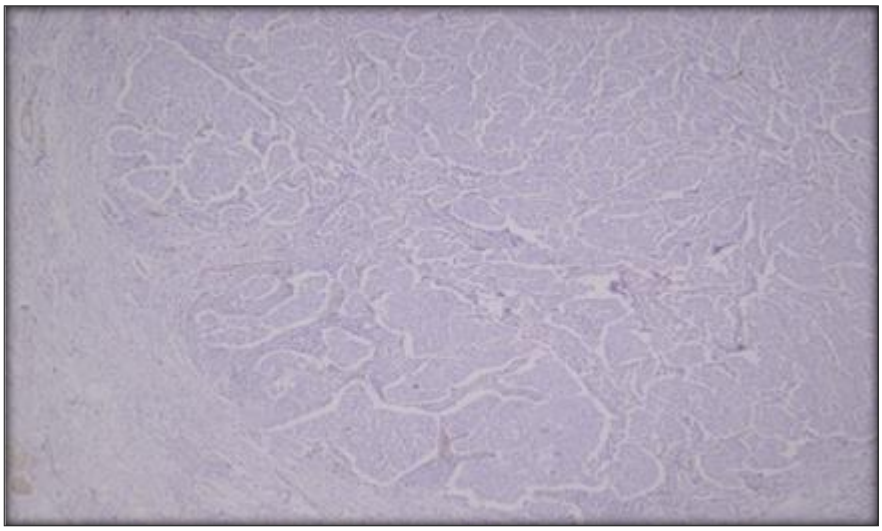


Figure 15: Breast carcinoma with medullary features (IHC x400) showing loss of CD34 expression

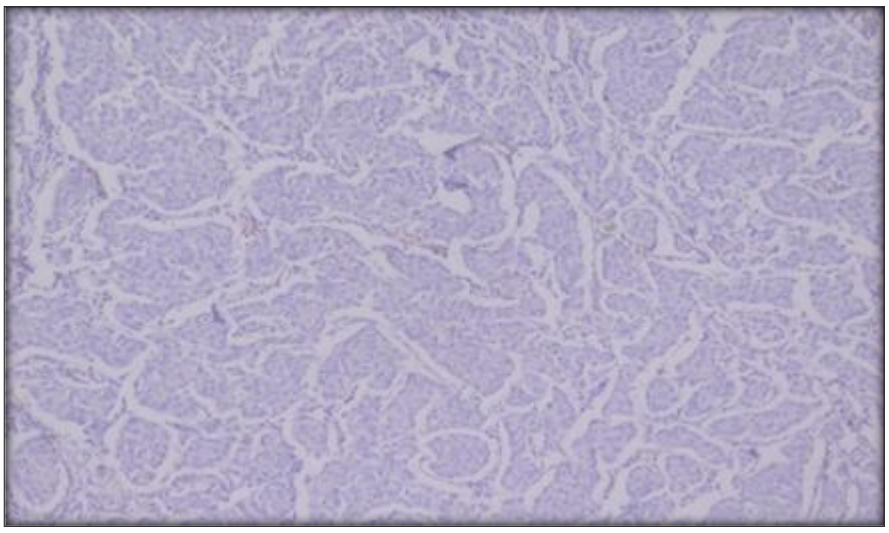


Figure 16: IDC with medullary features, IHC x400 showing loss of CD34 expression

ISSN: 0975-3583,0976-2833 VOL13, ISSUE 05, 2022

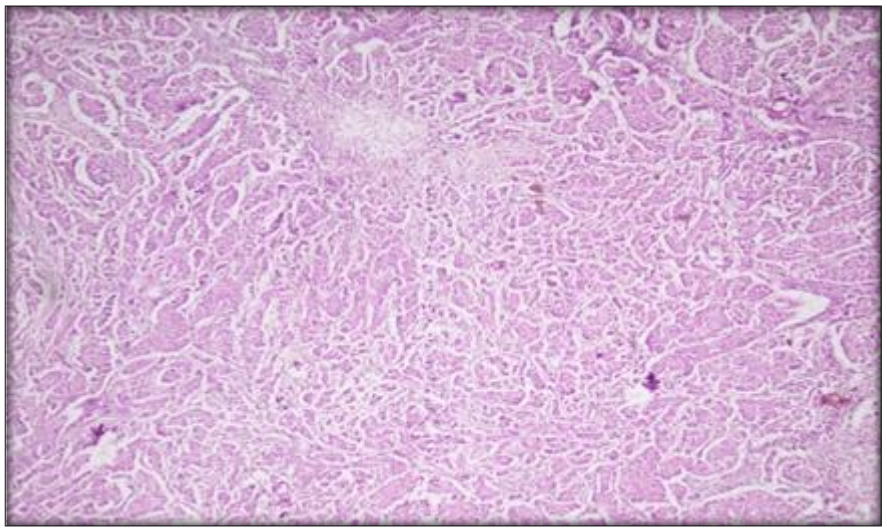


Figure 17: IDC with medullary features (H & E x40)

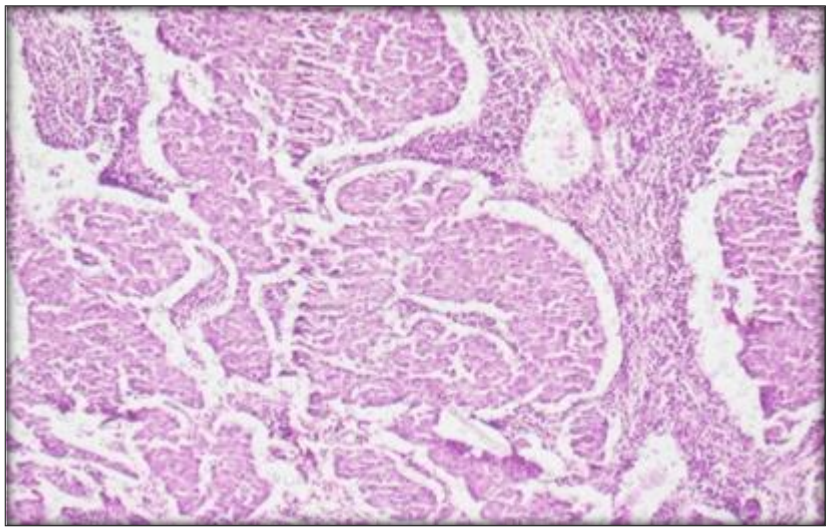


Figure 18: IDC with medullary features (H & E x400)

Discussion

Out of the 48 malignant cases, 97.9% cases had Grade 0 and 2.1% cases had Grade 2, i.e. majority of the cases (47) had complete loss of CD34 expression and a single case of DCIS had retained expression of CD34.This was consistent with studies done by Kuroda et al and Elancheran et al which showed 100% malignant cases in Grade 0 and the study by Khan et al which showed 97.9 % cases in Grade 0.All 39 cases of IDC, 3 cases of Invasive Lobular carcinoma, 2 cases of Mucinous carcinoma, 1 case of IDC with medullary features and 1 case of Micropapillary carcinoma showed Grade 0 on IHC. There were 2 cases of DCIS in the study, out of which 1 showed Grade 0 and the other showed Grade 2 on IHC.In the 5 cases of IDC, CD34 expression expression was seen in the adjacent stroma and in areas with increased vascularity.The comparison of CD34 expression with benign and malignant tumors was found to be highly statistically significant ($p<0.00001$).However, a few grey areas were seen like a case of Borderline PhyllodesTumour with retained expression and another case of DCIS with retained expression which were discordant with the findings of other authors. So, additional studies are needed to study the progressive loss or retention of expression in various lesions.Also, another limitation of present study was the use of a single marker. We

ISSN: 0975-3583,0976-2833 VOL13, ISSUE 05, 2022

conclude that CD34 marker can be used as an adjunct with other markers for better interpretation.

Conclusion

The significant differences in CD34 expression between benign and malignant breast lesions can be used to differentiate between the two. Moreover, it can be used as a diagnostic marker to study the progression of lesion. It can also be used as an adjunct with other markers already in use. However, further studies should be undertaken to establish its role as a therapeutic target in breast cancer lesions.

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ISSN: 0975-3583,0976-2833 VOL13, ISSUE 05, 2022

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