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IMMUNOHISTOCHEMICAL EVALUATION OF ANTIGENIC EXPRESSION OF CD34 IN BENIGN AND MALIGNANT BREAST LESIONS

Pathology

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ABSTRACT

Objectives: CD34 Immunohistochemical antigen serves as a tool in differentiating between benign and malignant conditions. Hence, the study was conducted to demonstrate the expression of CD34 antigen in stroma of benign breast lesions and to assess whether non expression of CD34 antigen is specific in stroma of malignant breast lesions.

Materials and methods: The present study was carried out in the Department of Pathology, Maharaja Agrasen Medical College from period of January 2020 to March 2021. Forty prospective cases of breast lesions were included in the study. All the forty cases were subjected to immunohistochemistry with CD34 antigen to study its stromal expression on benign and malignant breast lesions.

Results: On Hematoxylin and Eosin staining, 20 benign and 20 malignant cases were diagnosed. Benign cases included 11 fibroadenomas, 3 benign phyllodes tumour, and one case each of adenosis, fibroadenoma with moderate ductal hyperplasia, fibroadenoma with cystic and apocrine changes, fibroadenoma with ductal hyperplasia, simple epithelial hyperplasia and usual ductal hyperplasia. Malignant cases comprised of 18 invasive ductal carcinoma and one each of invasive lobular carcinoma and malignant phyllodes tumour.19(95%) of benign cases showed retained CD34 stromal expression, 1 benign phyllodes showed reduced expression. 19(95%) of malignant cases (invasive breast diseases) are showed loss of stromal expression (complete loss to reduced expression) while 1 malignant phyllodes showed retained expression.No significant correlation was found between CD34 expression and histological grade or lymph node metastasis.The metastatic foci in node positive patients showed a similar pattern of CD34 expression in the lymph nodes as those seen in the breast.

Conclusion: We can concluded that the use of CD34 Immunohistochemical marker positivity as an adjuvant tool in differentiating between benign and malignant conditions and can serve as an important diagnostic marker .

KEYWORDS

Breast, Stroma , CD34, Immunohistochemistry.

INTRODUCTION

A mass in the breast regardless of age and gender is a source of almost universal clinical concern with varying degrees of suspicion about a malignant process. The perinatal period, childhood and adolescence are important intervals for breast cancer risk development. Endogenous estrogen exposure is thought to be highest in utero and exposure to estrogen throughout life plays an important role in increasing breast cancer risk. Female breast cancer has now surpassed lung cancer as the leading cause of global cancer incidence in 2020, with an estimated 2.3 million new cases, representing 11.7% of all cancer cases. It is the fifth leading cause of cancer mortality worldwide, with 685,000 deaths. Among women, breast cancer accounts for 1 in 4 cancer cases and for 1 in 6 cancer deaths, ranking first for incidence in the vast majority of countries (159 of 185 countries).[2]

CD34, a 110-kDa transmembrane cell-surface glycoprotein, has been identified as a marker of human hematopoietic cells. Detailed structural analyses and cloning studies have confirmed that CD34 is a sialomucin, and have suggested that the fine composition of the carbohydrate moieties contained in its extended N-terminal region is important in determining its interactions with a variety of different ligands. The human CD34 gene is found on chromosome 1q32, a region containing several genes encoding adhesion matrix and complement cascade-binding molecules,such as L-selectin/P-selectin and E-selectin, laminin B2 ,and the RNA gene cluster.[3] CD34 is a specific marker of vascular endothelial cells and is particularly sensitive to tumour angiogenesis, as it can clearly represent the state of neovascularization during the growth of tumour.[4]

Normal mammary stroma contains a large number of CD34 positive fibroblasts which can be detected at the 10th week of gestation and in developmental phase these can be found in the majority of stromal cells. Normal breast shows CD34 positivity in both intralobular and interlobular stroma where the intralobular stromas has strong positivity, and interlobular stroma has weak positivity for CD34. The importance of stromal interaction with the ductal system is important for normal breast development and tumorigenesis in malignancy.[5] Endothelia showed a strong reactivity with CD34; no CD34 reactivity was observed with epithelial cells.[6]During the tumorigenesis, mutation in the premalignant ductal epithelial cells activated the adjacent stroma, and the activate stromal fibroblast undergoes 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 disseminate through the stroma because of the contractile property.[7] Tumour stromal interaction plays an important role in tumour invasion and metastasis of breast cancer.

The stroma around invasive breast tumours is known to differ from normal breast, with alterations in stromal protein synthesis and expression of MMP.[8] The term myofibroblast implies a homogeneous cell population, but there is increasing recognition of the heterogeneity of fibroblast populations, and it is therefore highly likely that not all myofibroblasts are the same, and there may be other aspects of the fibroblast phenotype that more closely reflect their functional differentiation.[9]

MATERIALS AND METHODS

Present study was conducted in the Department of Pathology in, Maharaja Agrasen Medical College, Agroha. All the biopsies/excision specimens of breast lesions received in Pathology Department for histopathological examination.Study period was of one year from january 2020 to march 2021.It was a prospective study, taking study period and resources for constraints into consideration, 40 cases of breast lesions were included in the study.The relevant clinical history of the patients was collected. Tissue sections recieved were assessed with Hematoxylin & Eosin staining after routine processing for histological type and representative paraffin blocks were chosen for immunohistochemical evaluation using CD34 antigen as per standard protocol.

Patients of all ages with benign and malignant breast lesions who underwent surgical treatment as primary modality of treatment was included in the study. Inadequate tissue samples, male breast lesions

gynaecomasia or carcinomas and follow up cases were not included. Stroma of the breast positive for CD34 expression was stained as brown in colour. Results are given based on morphology of breast and Immunohistochemical expression for CD34 which can be of potential diagnostic value in distinction between benign and malignant lesions of breast, as stroma in benign tissue stains positive for CD34 IHC and malignant cases show loss of CD34 staining. The staining of endothelial cells in blood vessels was taken as internal control.

Cd34 staining will be graded as 0, 1, 2, 3 as follows
<5% of CD34 positivity was considered as grade0,
5 to 25 % of CD34 positivity was considered as grade 1,
25 to 50 % of CD34 positivity was considered as grade 2,
>50% of CD34 positivity was considered as grade 3.

The following methods did a semi-quantitative assessment
Grade 0 – Interpreted as complete loss of CD34 Grade 1 – Interpreted as reduced expression Grade 2 and Grade 3 - Interpreted as a retained expression.[10]

The tests of significance used were chi square test, test of proportions and student's t test.

OSERVATIONS AND RESULTS

All the 40 cases included in the study were from female patients. Of these the youngest patient was of 10 years and oldest 75 years.Most of the benign cases are in age group 10-40yrs. Malignant cases are mostly in age group 31 to 60 yrs.12 out of 20 malignant cases (60%) are from left side of breast and 8(40%) from left side. 13 out of 20 benign cases (65%) are from right side of breast and 7(35%) are from left side. 75% malignant cases are from postmenopausal females and 85% benign cases are from premenopausal females, One benign case is taken of from a female who had not attained menarche.

Table 1: CD 34 grading in breast lesions as per histopathological diagnosis

Histopathological Diagnosis	Cd34 Grade				Total
	Grade 0	Grade I	Grade II	Grade III	
Adenosis	0	0	0	1	1
Benign Phyllodes Tumour	0	1	0	2	3
Fibroadenoma	0	0	2	9	11
Fibroadenoma with moderateductal hyperplasia	0	0	0	1	1
Fibroadenoma with cystic and apocrine change	0	0	1	0	1
Fibroadenoma with ductal hyperplasia	0	0	0	1	1
Invasive Ductal Carcinoma	11	7	0	0	18
Invasive Lobular Carcinoma	1	0	0	0	1
Malignant phyllodes tumor	0	0	1	0	1
Simple Epithelial Hyperplasia	0	0	0	1	1
Usual ductal hyperplasia	0	0	0	1	1
Total	12	8	4	16	40

As per CD34 grading out of 20 malignant cases, 18 Invasive ductal carcinoma cases are in grade 0, 7 in grade I. Invasive lobular carcinoma is of grade 0 and Malignant phyllodes tumour in grade II. Out of 20 benign cases 14 fibroadenomas are diagnosed, 11 are in grade III, 3 in grade II. Out of 3 Benign phyllodes tumour cases 2 are in grade III and one in grade I. Simple Epithelial Hyperplasia and Usual ductal hyperplasia are in grade III.

On comparison of the test group CD34 Interpretation with the Gold standard of HPE diagnosis the test group has a sensitivity of 95 % and specificity of 95%. The test has a positive predictive value of 95% and Negative predictive value of 95%. The test and the gold standard agree on 38 out of 40 having a diagnostic accuracy of 95%. The Kappa value of 0.9 indicates Excellent agreement with a p value of <0.001.

Sensitivity specificity of CD34 expression in predicting benign malignant

PARAMETER	TRUE NEGATIVE i.e HPE diagnosis : Benign & Test : Negative	TRUE Positive i.e HPE diagnosis : Malignant & Test : Positive	FALSE NEGATIVE i.e HPE diagnosis : Malignant & Test : Negative	FALSE POSITIVE i.e HPE diagnosis : Benign & Test : POSITIVE	SENSITIVITY	SPECIFICITY	POSITIVE PREDICTIVE VALUE	NEGATIVE PREDICTIVE VALUE	DIAGNOSTIC ACCURACY	GOLD STANDARD	KAPPA STATISTICS	P VALUE
CD34 Interpretation	19	19	1	1	95.00 %	95.00 %	95.00 %	95.00 %	95.00 %	HPE diagnosis	0.90 0 0	**<0.0 0 1**

DISCUSSION

Normal mammary stroma contains CD34 positive fibroblasts/ fibrocytes. The CD34 positive fibroblasts distribution is like that of normal skin. This is due to the development of mammary glands from solid epithelial cords growing from the epidermal layer into the underlying mesenchyme

Cd34 is a transmembrane glycoprotein that is involved in modulation of cell adhesion and signal transduction and is expressed in mesenchymal cells of the prostate, urinary bladder, fallopian tube, thyroid, pancreas, colon, uterine cervix and testis. On endothelial cells, CD34 acts as a ligand for L-selectin. They constitute a population of stromal cells that are capable of synthesising connective tissue matrix.CD34 positive stromal fibroblasts are potent antigen presenting cells, and they play a major role in host response to tissue damage. Loss of CD34 expression is noted in malignant transformation of mesenchymal cells in the stroma.[10]

In our study no significant correlation could be found between CD34 staining and either of the clinicopathological parameters, i.e., SBR grading (p value 0.828) or lymph node metastasis (p value 0.349). This was in contrast to the findings of Yazhou et al.[11]who compared the clinicopathological parameters between invasive ductal carcinomas with and without stromal myofibroblasts and revealed significant differences in lymph node metastasis and grade of differentiation.Our findings were however similar to those of Khan et al[8]and Catteau et al.[12] who also did not find any difference in CD34 expression between different histological (SBR) grades and lymph node status of IDC.

On the basis of stromal positivity CD34 grading is done according to neoplastic behaviour (benign and malignant) . 95% benign cases show CD34 grade 2(16%)& grade 3(80%) staining, while 100% invasive breast cancers including IDC and ILC show grade 0 (63%) and grade 1(37%) staining. 1 case of benign phyllodes show grade 1 and one case of malignant phyllodes show grade 2 staining. 19(95%) of benign cases are showing retained stromal expression, 1 benign phyllodes is showing reduced expression. 19(95%) of malignant cases(invasive breast diseases) are showing loss of stromal expression(complete loss to reduced expression) while 1 malignant phyllodes is showing retained expression.Hence difference in loss of CD34 expression between benign and malignant lesions overall, is highly significant statistically.

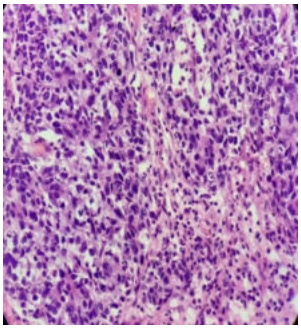

Figure 1: Invasive Ductal Carcinoma Breast. H&e (40x)

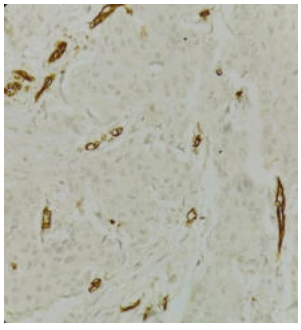

Figure 2: Invasive Ductal Carcinoma Breast. CD34 Immun Ostaining. Showing Complete Loss Of Stromal CD34 (Grade 0). Only Blood Vessels Seen. (40x)

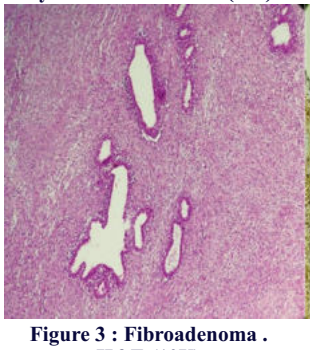

Figure 3 : Fibroadenoma . H&E (10X)

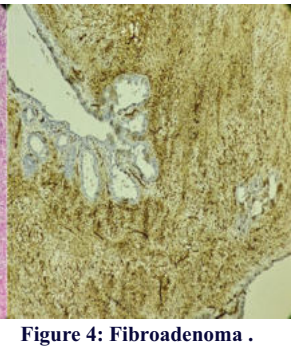

Figure 4: Fibroadenoma . CD34 immunostaining showing retained expression (Grade 3). (10X)

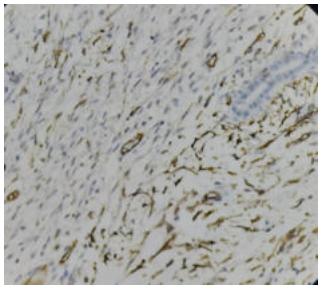

Figure 5: Malignant phyllodes tumour breast. CD34 immuno staining. Showing patchy expression of stromal CD34 (grade 2). (40X)

Most of the benign lesions in the present study showed a CD34 retained expression with dense, diffuse staining for CD34 in the stroma (grade 2 to 3). Similar results came in studies done by Khan et al[8], Barth et al[13], Catteau et al[12] and Chauhan et al.[14]The previous researchers in this field have shown loss of CD34 expression in cases of breast malignancies. However, there are certain controversies regarding the extent of loss of CD34 in case of IDC and ILC. While Catteau et al[12] and Cimpean et al[15] proposed that infiltrative lobular carcinoma shows less marked loss of CD34 as compared to IDC.Our view on the subject is similar to that of Khan et al[11] and Kuroda et al[16] as the frequency of loss of CD34 in cases of IDC and ILC was very similar in present study.Cimpean et al[15] and Chauhan et al[14] show similar loss of CD34 in malignant breast lesions specifically invasive breast diseases as in present study.In contrast to the present study Khan et al[8], Kacar et al[17] and Cimpean et al[15] showthat malignant phyllodes tumour of the breast exhibit lower levels of CD34 expression than benign phyllodes tumour.

CONCLUSION

The present study shows the expression of CD34 in stroma is retained in most of the benign breast lesions and stromal expression of CD34 is almost completely lost in all malignant neoplasms especially the invasive breast diseases. The present results indicate that CD34 positive stromal cells were consistently lost in the stroma of invasive carcinoma of the breast and detection of a large number of CD34-positive stromal cells in the stroma of a breast lesion strongly suggests that the lesion is benign. In this study Phyllodes tumour has shown indolent behaviour as one malignant case had retained expression and one benign case had reduced expression, so further studies with more phyllodes cases are needed to study CD34 stromal expression in phyllodes tumour.Owing to the significant differences in CD34 stromal expression between benign and malignant breast lesions, it can potentially be used to differentiate between the two and can serve as an important diagnostic marker. This potential for the stromal response to the tumour is becoming a target for the treatment of breast malignancy. Further studies can also be undertaken to establish its role as a therapeutic target in cases of breast cancer.

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