



Study of CD117 Expression on Phyllodes Tumor of Breast and Its Correlation with Histopathological Grade

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Abstract: Background: Accurately predicting the nature and differentiating of various grades of Phyllodes tumor, the diagnosis may vary by different pathologists¹, as there is no standard interpretation of the histologic cut-point criteria and therefore. In this study, we evaluated the impact of CD117 expression on Phyllodes tumor of breast and usefulness of this marker to differentiating benign from malignant phyllodes tumors. **Methods:** This cross-sectional observational study was conducted in the Department of Pathology, Dhaka Medical College. The study includes mastectomy/lumpectomy specimens received from surgery and surgical oncology departments and diagnosed as phyllodes tumor histopathological. Tissue processing and staining were performed using standard protocol. Histopathological typing and grading were done according to WHO recommended criteria 2012. Immunostaining for CD117 done on all 50 cases. Toluidine blue staining was performed on CD117 immunostaining positive cases, to see presence of mast cells. **Result:** This study consists of 50 cases of phyllodes tumor of breast. In all the cases immunohistochemistry was done with markers CD117. Histological Grading of phyllodes tumor was done using stromal cellularity, nuclear pleomorphic, stromal overgrowth, mitotic rate, margin of tumor. Statistical analysis was done and results were compared with various available previous studies. This study showed that most of benign/low grade phyllodes tumors belonged to the age group of 35-46 years and most of malignant phyllodes tumors belonged to the age group of 30.50-47.50 malignant phyllodes tumor in this study was 40 years. Overall 44% (22 out of 50) of the cases showed positivity for CD117 in the stroma. Among them 85% cases of malignant Phyllodes tumors of breast shows stromal expression of CD117. This study shows association of CD117 expression with malignant phyllodes tumor. Epithelial CD117 positivity was found in 69.5% cases of benign/low grade Phyllodes tumors of breast. Current study also shows association of CD117 epithelial expression with benign phyllodes tumor the result of our study show strong correlation between

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CD117 expression and histopathological grade. **Conclusion:** In conclusion, we can report an association of CD117 protein expression in borderline/malignant tumors and which may be associated with worse prognosis. So we can suggest for targeted therapy for these patient group having Phyllodes Tumor with positive CD117 expression.

Keywords: CD117 expression, phyllodes tumor, prognostic assessment, c-kit, recurrence.

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INTRODUCTION

Mammary phyllodes tumor (MPT) is a specific variant of breast tumor with benign (low grade) borderline and malignant features. It contains two predominant stromal and parenchymal parts of the tumor, thus phyllodes tumor is classified as biphasic tumor [1].

It's consisting of 0.3% -1% of all tumors that represent up to 2.5% of all fibro adenomatous breast lesions [2].

In Asian countries, phyllodes tumor occurs at younger age (average 25-30 years) [3]. However, an incidence rate of 6.92% was reported in a Singaporean study, suggesting its higher frequency among Asian women [4]. In the literature, the recurrence rate of Phyllodes tumor ranges from 10-25% for benign phyllodes, up to 32% for borderline Phyllodes and up to 40% for malignant Phyllodes tumor [5].

Treatment is mainly surgical, wide local excision with clear margins or mastectomy [6]. However role of adjuvant chemotherapy and radiotherapy is not well established [7]. Several biomarkers have been reported to be associated with histologic grades and show some prognostic value. However, at present, none of them have been proven to be of clinical value in daily practice. There are a few studies using immunohistochemically stains such as p53 [8, 9], CD34 [10], Actin [11] c-kit (CD117) [12] and CD10 [13] to correlate with the grading of Phyllodes tumor, but none of them have been proved to be clinical value in daily practice.

In recent years, the role of c-kit in the development of pre invasive and invasive breast carcinomas has been investigated [14]. It is established that C-kit mutations play an important role in the development of gastrointestinal stromal tumors (GIST), chronic myeloid leukemia (CML) and other neoplasms. Imatinib has shown to be effective in the treatment of Chronic Myeloid Leukemia (CML) and Gastrointestinal Stromal Tumor (GIST), which express high positivity of c-kit. Studies show that c-

kit expression is found in 50-100% of Malignant Phyllodes tumors by utilizing immunohistochemistry and targeted therapy may be successful mode of treatment in cases of MPT.

In this study, we evaluated the impact of CD117 expression on Phyllodes tumor of breast and usefulness of this marker to differentiating benign from malignant phyllodes tumors.

OBJECTIVES

General Objective

1. The objective of this study is to, "Evaluate the expression of c-kit (CD-117) immunohistochemical (IHC) expression in Phyllodes tumors of breast in patients presenting to tertiary care hospital."

Specific Objectives

1. To evaluate the relative frequency of different types of phyllodes.
2. To co-relate the relationship between pathologic type of tumor with age, size and presence or absence of expression of CD 117.
3. To assess the usefulness of these markers in differentiating benign from malignant phyllodes tumor of breast for appropriate treatment can be administered.

MATERIALS & METHODS

This was a cross-sectional, observational study was carried out at the Department of Pathology, Dhaka medical college, from January 2018 to December 2019. Based on the estimated frequency of 50% a sample size of total of 50 cases was enrolled in this study. Bound to the error of estimation of 15% or more and a confidence interval 95%

$$n = \frac{z^2 (pXq)}{d^2}$$

The study includes mastectomy/lumpectomy specimens received from surgery and surgical oncology departments and diagnosed as phyllodes tumor histopathologic ally. Tissue

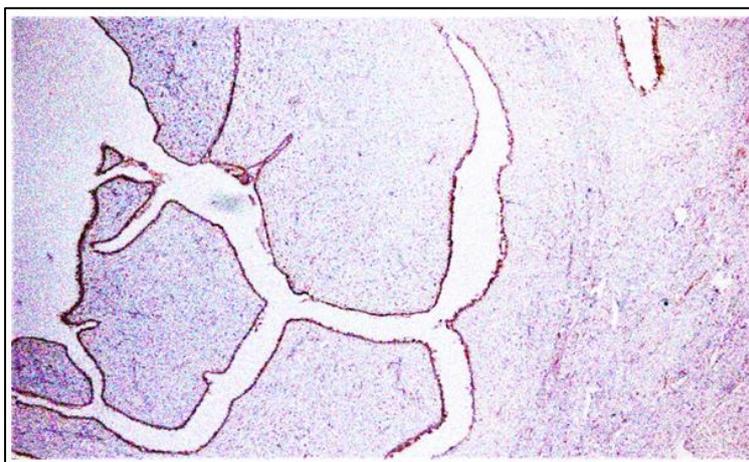
processing and staining were performed using standard protocol. Histopathological typing and grading were done according to WHO recommended criteria 2012. Immunostaining for CD117 done on all 50 cases. Toluidine blue staining was performed on CD117 immunostaining positive cases, to see presence of mast cells.

Staining pattern of CD 117 is both cytoplasmic and membranous. Intensity of staining of stromal cells for CD 117 will be assessed using cytoplasmic staining of breast epithelium as internal control. Staining is graded based on intensity of staining and percentage of spindle cells that took up the stain. Scoring criteria 1% cutoff is generally used because of the fact that CD117 is not expressed normally in breast stromal cells and even a low percentage of the protein expression can be an indicator of an abnormal state [7].

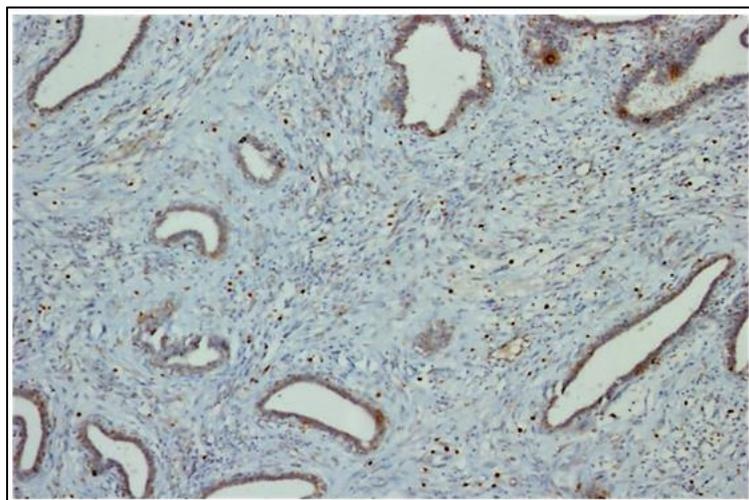
Staining intensity: 0-Absent, 1+ Weak, 2+ moderate; and 3+ Strong. Percentage of positive

tumor cells: 0, 0% of cells positive; 1+, 1–25% positive; 2+, 25–50% positive; and 3+, 50–100% positive. Score 0 was count as negative whereas Score 1, 2, and 3 were considered as positive.

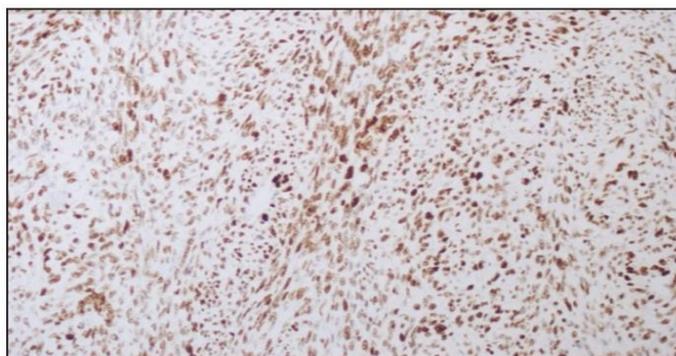
Microscopy of the slides was done and photographed at 200× magnification utilizing a digital microscope camera (Olympus AX80 DP21; Olympus, Tokyo, Japan) interfaced with a computer (Figure 1A, 2B). Statistical analysis of the result was obtained by window based computer software devised with Statistical Packages for Social Science version 19 (SPSS-19).ANOVA with *post hoc* comparisons was used to determine differences in patient ages and tumor sizes between benign, borderline malignant and frankly malignant phyllodes tumors. X^2 test and X^2 test for trend were used to determine the difference between the expressions of c-kit among the three categories of phyllodes tumors. The statistically significant level was set at 5%.



A



B



C

Figure 1: CD117 expression according to tumor type. (A) Microphotograph of benign phyllodes tumor with mild (+1) expression of CD117. (B) Microphotograph of Borderline phyllodes tumor with moderate (+2) expression of CD117. (C) Microphotograph of malignant phyllodes tumor with strong (+3) expression of CD117

RESULTS AND OBSERVATION

A total number of fifty diagnosed case of phyllodes tumor of breast were included in this study. Supplied specimens were collected by various surgical procedures e.g. like mastectomy,

lumpectomy etc. After histopathological diagnosis, immune histochemical study of CD 117 was performed and correlation with grading and staging was done.

Table 1: Clinical pathological characteristics of phyllodes tumors in association with CD117 stromal positivity on 50 study cases

Clinicopathological parameters	CD117		CD117		P-value
	Positive	(%)	Negative	(%)	
Age (Mean 40 years, median 42 years , range 15-60)					0.25 ^{ns}
≤ 40 years	12	24	09	18	
>40 years	13	26	16	32	
Tumor size (Mean 6cm, median 5cm, range 1.5-20cm)					0.5 ^{ns}
≤6 cm	13	26	17	34	
>6 cm	14	28	06	12	
Tumor Grade					0.016 ^s
Low grade	03	06	20	40	
Border line	08	16	06	12	
Malignant	11	22	02	04	
Stromal cellularity					0.016 ^s
Mild	04	08	17	34	
Moderate	09	18	05	10	
Marked	13	26	02	04	
Nuclear Pleomorphism					0.016 ^s
Mild	04	08	17	34	
Moderate	09	18	05	10	
Marked	13	26	02	04	
Stromal overgrowth					0.016 ^{ns}
Mild	04	08	17	34	
Moderate	09	18	05	10	
Marked	13	26	02	04	
Mitotic Activity					0.008 ^s
0-4	06	12	17	34	
5-9	09	18	07	14	
≥10	11	22	00	00	
Margin					0.8 ^{ns}
Pushing Border	10	20	19	38	
Infiltrating Border	13	26	05	10	
Site of Breast					0.25 ^{ns}
Right	14	28	11	22	
Left	10	20	13	26	

Chi-Square test done to measure the level of significance
 Ns =Non-significant
 s=significant

Here mean age were taken 40 years and 21 cases were belong to ≤ 40 years and 29 cases were >40 years. Average Tumor size were 6 cm , where 30

cases were found ≤ 6cm and 20 cases were below 6cm of tumor size .Stromal cellularity , nuclear pleomorphic, mitosis and tumor margin statistically significant ($p < 0.05$) and correlation CD117 expression were showed .Mitotic activity also correlated with CD117 expression and statistically significant ($p < 0.05$).

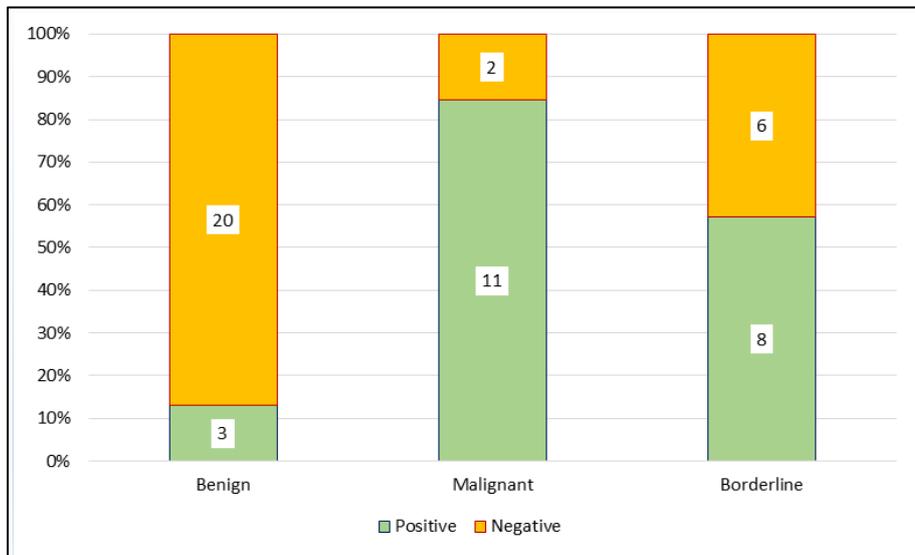


Figure 2: Association of Stromal expression of CD117 with Histopathological Grade (Here n=50, $p < 0.05$)

Chi-Square test done to measure the level of significance
 n= case no
 P value

Overall 85% of malignant tumors (11/13cases) showed stromal positivity for CD117 and 57% of borderline tumors showed stromal expression of CD117. About 14% of benign cases

showed stromal positivity for CD117. The association of stromal expression of CD117 with malignant and borderline tumors is statistically significant, p value (0.001) is less than 0.05 (Chi-square test). Overall 13 cases of malignant tumors 3 cases showed epithelial positivity for CD117 and 14 cases of borderline tumors, 9 cases showed stromal expression of CD117. About 23 cases of Low grade, 16 cases shows epithelial positivity for CD117.

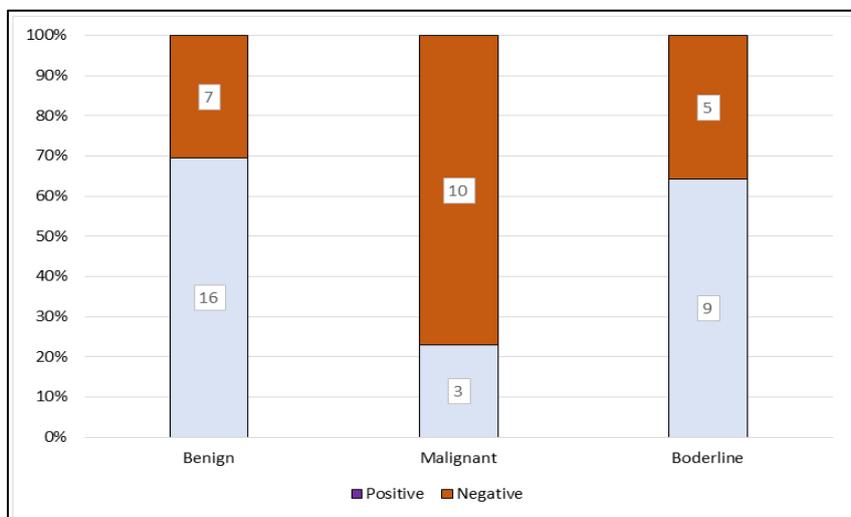


Figure 3: Association of distribution epithelial expression of CD117 with Histopathological grade of study cases

Chi-Square test done to measure the level of significance.

About 70% cases of benign PTs, 64 % of cases of borderline PTs and 13% cases malignant PTs showed stromal positivity for CD117 immune expression. The association of epithelial expression of CD117 with benign tumors is statistically not significant, p value (0.1) is less than 0.05 (Chi-square test).

DISCUSSION

Breast phyllodes tumors (PT) can present as benign, borderline or malignant neoplasm but clinical behavior is difficult to predict on histology and. There is limited amount of data available on the role of biological markers. The c-kit gene encodes a transmembrane tyrosine kinase receptor protein. In the breast, it has been found that C-kit expressed in the normal breast ductal epithelium and myoepithelial cells, with reduced expression in benign breast lesions and carcinoma. (Natali PG *et al*, 1992)

Current study the age range of study population was 15-60 years (mean age was 40.23±1 years). Most of the population was distributed in between 35 to 56 years age group. This correlate with Huang KT *et al*, [18] and Tan PH *et al*, [4] showed age distribution between 16 to 69 years (median, 42 years). This is observation consistent with current study.

This study classify all the cases according proposed by the World Health Organization (WHO) 2012 criteria, that is stromal cellularity, cellular pleomorphic, mitotic activity, margin appearance and stromal distribution. Total 50 cases phyllodes tumor of our study, 23 (46%) cases was low grade, 14 (28%) cases were border line and 13 (26%) cases were malignant PTs.

With reference to CD117 expression, most of the malignant phyllodes tumor shows stromal positivity that is 11(87%) cases out of 13 malignant PTs. It also observe C kit expression also notice in some case of benign (14%) and borderline case (57%) PTs. Degree of CD117 expression increase as degree of malignancy increase. Chen *et al*, [17] and Sawyer *et al*, [12], found stromal c-kit expression on malignant tumors. This finding were consistent with current analysis which also statistically significant.

Current study show also shows association with CD 117 expression with mitotic activity. We found that within 0-4/10 HPF mitotic rate, total 17 cases PTs were identified, among them 6(17.3%)

cases were positive for CD 117 immune expression and all the cases were low grade. Again mitotic activity $\geq 10/10$ HPF we found 11 cases PTs, all were malignant PTs, showed 100% expression of CD117. Here show that increasing the mitotic activity stromal expression of CD117 also increasing. The association of stromal expression of CD117 with mitotic activity was statistically significant. Wai J T *et al*, [7], 2014 shows increase percentage of CD 117 expression with increase of stromal mitosis.

However, there were also several studies indicated no association between CD117 expression and tumor grade. Djodjervic B *et al*, 2008 [18], had a study of CD117 expression, in phyllodes tumors on 68 cases. He do toluidine blue staining on CD117 positive case and showed the staining pattern matched that of c-kit in the number of cells and their distribution, thereby he confirming that presence of mast cells and excluding any appreciable level of true stromal c-kit staining.

In our study we observed CD117 expression mostly in borderline and malignant PTs. All the positive cases of CD117 was perform Toluidine blue staining to rule out possible confounding contribution of mast cells, as previously suggested by Djodjervic *et al*, 2008 [18]. Current study shows Toluidine blue negative on cases that we defined as CD117 positive. So our initial observations reinforced.

We found some literature work on CD 117 expression on recurrent case like, Tan puay-hoon *et al*, [4], and Wai J T *et al*, 2005 [7] work show, the case which are showing stromal reactivity for CD117 being more likely to recur than those that were negative. Although the general trend of increasing staining was observed with increasing degree of malignancy, So, all the study shows c-kit (CD117) is associated borderline PTs and frankly malignant PTs and we also observe in our current study. So we can consider CD117 expressionism a prognostic indicator for Phyllodes tumor.

However, the higher rate of c-kit expression in malignant phyllodes tumors in our study can be view of the action of the newly developed targeted therapy. Which inhibits tyrosine kinase receptors, and its usefulness in gastrointestinal stromal tumors, this drug may theoretically also be useful in the management of malignant phyllodes tumor, particularly with tumor recurrences or distant metastases.

Our study limited owing to small sample size, single institute data and no recurrence disease

cases .According to published literature , studies have showed that CD117 expression increase from benign to malignant tumors but few studies deny that , so there is need of new resurrect in this aspect to establish strong relationship between CD117 & and PT of breast .

CONCLUSION

In this study, we demonstrated increasing c-kit expression in mammary phyllodes tumors, with increasing degree of tumor grade and can act as prognostic factor for phyllodes tumor. The new therapeutic agent targeted therapy, may be a useful drug therapy for this disease, particularly in the tumor recurrences and advanced-stage disease.

Our findings show that CD117 markers are differentially expressed in benign and malignant PTs. CD117 was preferentially expressed in the stroma of malignant PTs. Our results suggest that these marker might be used for the diagnosing the various histopathological grades of PT. In Bangladesh no study was conducted in CD117expression in phyllodes tumor and correlation with its staging and grading .A larger study of PTs will be useful to accurately evaluate the significance of immunohistochemically markers with respect to histological grade and clinical outcome and to further understand the biological behavior and tumor progression and effectiveness in treatment Phyllodes Tumors.

Human Ethics

Consent was obtained or waived by all participants in this study. Ethics Review Committee Dhaka Medical college issued approval 13/06/2019. The study was approved by Ethics Review Committee Dhaka Medical College.

Animal Ethics

Animal subjects: All authors have confirmed that this study did not involve animal subjects or tissue.

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