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Diagnostic and Prognostic Role of Stromal CD 10 and Ki 67 in Benign and Malignant Phylloides Tumor of Breast

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Abstract

Background: Phylloides tumors (PT) are rare fibro epithelial tumor of breast, closely resemble to breast fibroadenoma, ranging from benign to malignant tumor. PT has potential to recur and metastasize due to its stromal characteristics. Differentiation between benign and malignant tumor is not possible with histopathology alone, additional study of stromal CD 10 and Ki 67 antigen expression in PT may be useful to differentiate benign from malignant tumor.

Method: Immunohistochemical study using specific monoclonal antibody to label stromal CD 10 and Ki 67 antigen were done in tissue sample of 35 patients.

Result: Out of total 35 patients histopathologically 18 were benign, 9 borderline and 8 were malignant PT. On immunohistochemistry 16 patients had CD 10 expression labeled as CD 10 positive (7 malignant, 7 borderline and 2 benign), and 19 were CD 10 negative (1 malignant, 2 borderline and 16 benign) ($p< 0.000$). Overall sensitivity, specificity, PPV and NPV to diagnose malignant PT was 82.35%, 88.88%, 77.77% and 94.11% respectively.

23 patients had Ki 67 labeling index between 1-8 (18 benign, 5 border line) and 12 patients had index > 8 (8 malignant, 4 borderline) i.e. no one malignant PT had Ki 67 labeling index < 8 ($p <0.000$). Sensitivity, specificity, PPV and NPV for diagnosing malignant PT were 100%.

Conclusion: Stromal CD 10 and Ki67 expression is higher in malignant PT. Ki 67 labeling index >8 are strongly suggestive of malignant PT and needs to appropriate treatment and close follow-up even in histopathologically benign tumor.

Keywords: Phylloides tumor; CD 10, Ki 67; Immunohistochemistry; Histopathology

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Introduction

Phylloides tumors (PT) are unique in combining sarcoma with a benign epithelial component, having varying malignant potential ranging from benign to completely malignant tumor. PT is a uncommon fibroepithelial tumor of breast, accounting 0.3% -0.5% of all breast neoplasm with incidence of 2.1 per million. Age of presentation is one decade earlier to breast carcinoma and one decade later to fibro adenoma with peak age of 45 to 49 years [1,2]. On the basis of histological behavior WHO classified PT into, benign, borderline malignant and malignant category [3]. Distant metastasis is rare but local recurrence can occur uncommonly which is not related to histological behavior of tumor as benign tumors can also recur [4-6].

Diagnosis of phylloides tumor can be difficult on histopathological characteristics alone because it closely resemble to fibroadenoma [7] therefore an additional study of genetic changes that characterize the course of Phylloides tumor may have additional prognostic significance. Advances in immunohistochemical and molecular methods have shed light on the biological nature of the neoplasm. Molecular markers have been extensively investigated with a view to providing early and accurate information on long-term outcome and prediction of response to treatment of early breast cancer.

Ki 67 is a proliferation marker, its expression are considered useful predictor of tumor progression and cancer prognosis. In this study we also study CD 10 is a member of the family of matrix metalloproteases which in general, function to degrade matrix adhesions rendering invasive and metastatic property to any neoplasm. To date very few study correlated the expression of Ki 67 with CD 10 and also correlation of these markers with histopathological grading and with other clinical parameters.

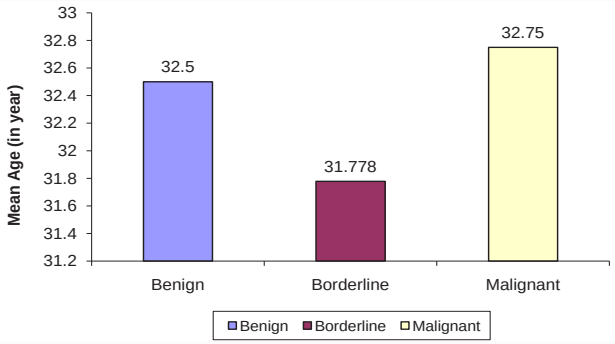

Figure 1: Mean age of patients categorized as per Histopahological behavior.

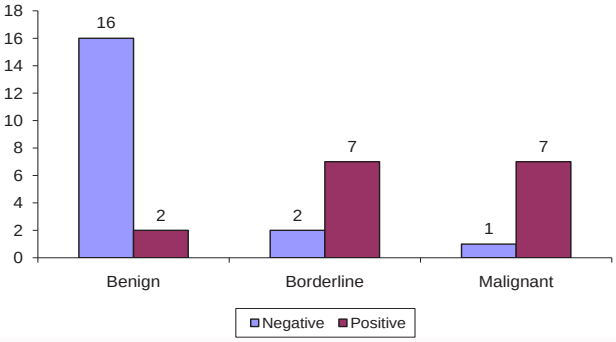

Figure 2: Diagrammatic presentation of CD 10 expression in benign, borderline and malignant phylloides tumor.

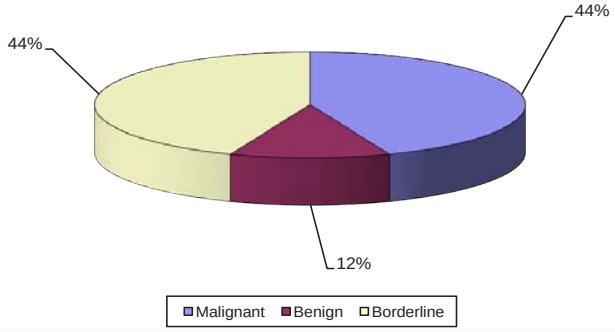

Figure 3: Pie chart representing expression of CD 10 positive cases.

Aims and objectives

The aim of study is to evaluate the difference of immunophenotype of the Stromal cells and cells proliferation marker in the Phylloides tumors, and to evaluate the differential feature between benign and malignant Phylloides tumors. In this study we compare diagnostic and prognostic role of Stromal CD10 and Ki67 and expression in benign and malignant Phylloides tumor of breast and correlate with known clinical and histopathologically prognostic factors.

Material and Methods

Paraffin fixed tissue samples of 35 patients were sent for histopathological and Immunohistochemical study, where hematoxylin and eosin staining slides prepared and histopathological study were performed. Histopathologically diagnosed benign, borderline and malignant PT of breast was taken. Positive tissue control (Fibroadenoma for CD 10 and Lymphoma for Ki 67) and negative control (Omission of primary antibody) were taken.

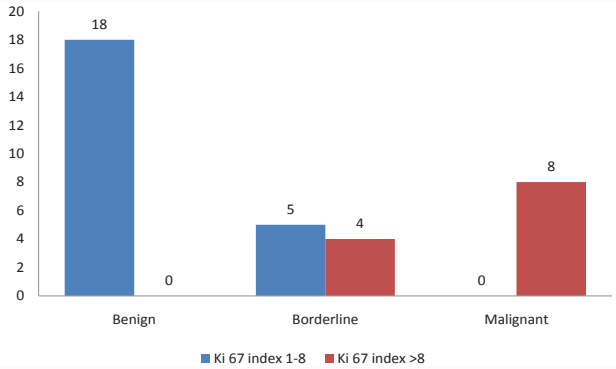

Figure 4: Bar diagram showing Ki 67 index.

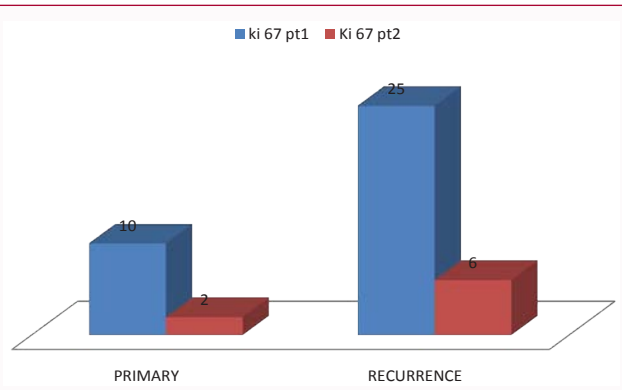

Figure 5: Expression of Ki 67 in Patients of Phylloides Tumor with Recurrence.

For Immunohistochemistry Streptavidin Biotin immunoperoxidase method was done. Staining and evaluation using specific monoclonal antibody to CD 10 and Ki 67 was done as per standard protocol, the tumor was considered positive for CD 10, if the Stromal cells were moderate to strong staining intensity in 20% or more of the Stromal cells.

Ki 67 labeling index is defined as the percentage of cells that showed a positive nuclear stain. At least 1000 stromal cells were counted for this analysis, and all Ki 67 indices were determined by one observer.

Statistical analysis and data Interpretation

For data interpretation appropriate statistical test like chi-square, ANOVA, Fischer`s `t` Test has been considered and results were drawn on the basis thorough statistical analysis using SPSS 16.0 (IBM) software.

Observation and results

Following results were obtained from study, out of 35 patients 18 were benign, 9 were borderline malignant and 8 were malignant phylloides tumor. . The mean age of patient of phylloides tumor was 32.37 years, with a range being 13 to 53 years. No significant differences between mean age (32.5 vs. 31.778 vs. 32.75, p >0.05) of benign, borderline malignant and malignant Phylloides tumor was noted.

Out of 16 CD 10 positive patients seven were of malignant histology (7/16, 44%), seven were of borderline (7/16, 44%), and only 12% of the patients were diagnosed as benign on histopathology examination (p=0.000). Over all sensitivity and specificity of CD 10

Table 1: Descriptive analysis of age of different histopathological groups of phylloides tumor (1 Benign, 2 Borderline malignant, 3 Malignant).

Age (year)	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
1	18	32.500	9.6360	2.2712	27.708	37.292	13.0	44.0
2	9	31.778	7.1899	2.3966	26.251	37.304	18.0	40.0
3	8	32.750	9.1456	3.2335	25.104	40.396	25.0	53.0
Total	35	32.371	8.7147	1.4731	29.378	35.365	13.0	53.0

F-value= 0.029, p = 0.972

Table 2: Expression of CD 10 out of 35 patients 16 patients had CD 10 expression were labeled as CD 10 Positive, 19 patients failed to express CD 10 were labeled as CD10 Negative.

Histopathology	CD10		Total
	Negative	Positive	
Benign	16	2	18
Borderline	2	7	9
Malignant	1	7	8
Total	**19**	**16**	**35**

Pearson Chi-Square 18.042, p=0.000.

to diagnose malignant potential was 82.35 % and 88.88% respectively, however independent sensitivity and specificity of CD 10 for assessing malignant potential of tumor 87.50% and 88.88% respectively. Diagnostic accuracy of test for combined borderline and malignant tumor was 85.71%, while only for malignant tumor was 88.46%. PPV is 77.77% and NPV 94.11 % observed in our study.

Out of 35 patients 23 patients had Ki 67 index between 1-8 (benign 18, borderline 5), and 12 patients had Ki 67 index >8 (borderline 4, malignant 8). Patients of benign phylloides tumor had Ki 67 proliferation index < 8 as compared to malignant and borderline malignant tumor (p = 0.000), as similarly malignant phylloides tumor had Ki 67 proliferation index >8 as compared to benign histology (p = 0.000)). Sensitivity and Specificity of test for malignant phylloides tumor alone were found 100%. Fisher exact `t` test did not showed significant difference (p >0.05) in between CD10 and Ki 67 for diagnosing a malignant phylloides tumor.

As the diameter of phylloides tumor increases positivity of CD-10 expression increases. (p< 0.05). Out of 16 CD 10 positive cases only 4 patients had a longest diameter of tumor < 5 cm, while 7 out of 19 CD 10 negative cases have diameter >5 cm. Analytical study did not show linear association between Ki 67 Index and longest diameter of phylloides tumor (p >0.05).

No relationship was observed in our study between palpable lymph nodes and expression of Immunohistochemical marker CD 10 and Ki 67 (p >0.05). No metastasis was found in any of our patients.

In 3 recurrent cases included in our study the CD 10 expression remained same in both primary and recurrent diseases, while Ki 67 expression increased in recurrent tumors as compared to their primary counterpart; however a sample size of two or three patients is too small to make a universally acceptable hypothesis and to formulate treatment guidelines.

Table 4: Expression of Ki 67 malignant Vs. Benign.

	Malignant	Benign
Ki-67 Index 1 – 8	0	18
Ki-67 Index > 8	8	0

Table 5: Expression of Ki 67 in Patients of Phylloides Tumor with Recurrence.

Patient	Ki 67 Index	
	Primary Case	Recurrence Case
1	10	25
2	2	6

Those two patients who present with recurrence , primary tumor is treated by local excision and one patient whose primary record not available, all these three patients with recurrence are now treated with modified radical mastectomy. There is neither history, nor any metastatic work-up like USG ABDOMEN, X-RAY, and LFT suggestive of distant metastasis present in all 35 patients of phylloides tumor.

Discussion

Phylloides tumors are rare neoplasm that may poses difficulty in predicting the clinical outcome on merely histological evaluation of tumour tissue**. WHO** [3] and Azzopardi and Salvadori et al. [1,8] classified phylloides tumor into benign, borderline malignant and malignant groups on the basis of histological features. Hart et al and Kim et al. [9,10] preferred benign and malignant without borderline type. Azzopardi et al**.** [1] suggested that there is no difference between borderline and malignant tumor in terms of local and distant relapse and the same hypothesis was also followed in our study however, local recurrence may occur in all categories of phylloides tumor [4].

CD 10 is a zinc dependent metalloproteinase that cleave the protein component of extracellular matrix and play a major role in tissue remodeling. Being a member of metalloproteinase family it is possible that increased CD 10 expression may facilitate the metastatic potential of a tumor and may also potentiate the vascular invasion [11].

Table 3: Published Reports of Ki 67 Proliferation Index.

Author	% Of Positive Cells	Benign	Borderline	Malignant
Gatalica [13]	Ki 67 mean %	7.73	NA	23.42
Kleer [14]	Ki 67 mean %	3.6%	16%	50%
Kocova [15]	Ki 67 mean %	4.7%	NA	15.4%
Kuenen [16]	Ki 67 > 20%	10%	37.5%	100%
Niezabitowski [17]	Ki 67 > 11.2%	4%	17%	52%

One of the largest Study done by Tse et al. [12] from China in 181patients of phylloides tumor using positive staining of stromal CD 10 as a diagnostic criteria showed 5.88% (6/102) to be benign, 31.37% (16/32) as borderline and 50% (14/28) as malignant phylloides tumor. The study overall had a specificity of 95%, positive productive value (PPV) of 81%, sensitivity of 38%, negative productive value (NPV) 72% and an accuracy of 74%.

Similar results have been found in our study in which only 2 out of 18 benign phylloides tumor were reactive (11.11%), seven out of 9 borderline patients were CD 10 positive (77.77%), while from 8 malignant phylloides tumor cases seven were CD10 positive (87.5%). The CD 10 expression as a diagnostic test had a sensitivity of 82.35 % for borderline malignant and malignant tumor taken together and a sensitivity of 87.5 % for malignant tumor alone. The Specificity being 88.88% in both borderline malignant and malignant taken combindly and in considering malignant tumor alone i.e. excluding borderline tumor. Diagnostic accuracy of test for combined borderline and malignant tumor was 85.71% and 88.46 % for malignant tumor alone. The PPV and NPV were 77.77% and 94.11 % respectively. CD 10 expression was significantly high in case of malignant phylloides tumor as compared to benign ($p< 0.000$). Similarly CD 10 expression was significantly high in larger tumors as compare to smaller one ($p< 0.05$) with eight of ten patient with diameter >10 cm tested positive for CD 10,while only 4 of 11 patients being positive in tumors less then 5cms in size. Similar association of CD 10 expression with increasing age has been observed by Tse et al [12].

Ki 67 is a cell proliferation related protein that can be labeled with monoclonal antibody MIB-1, also known as MK 167. The Ki 67 labeling index is the number of cells that express immunostaining for Ki 67. Its expression is present in active phases of cells and absent in resting cells. The fraction of Ki 67 positive tumor cells i.e. Ki 67 labeling index is often correlated with the clinical course of cancer.

Several published series show that expression of proliferative antigen Ki 67 when correlated to tumor grading had a higher Ki 67 index in malignant tumor as compared to benign histology. Noronha et al. [13] of Loma Linda, USA study to evaluate the expression of CD34, CD117 (c-kit), and Ki-67 in PT of the breast and attempt to correlate the staining pattern with tumor grade by morphology. Although most benign PTs (80.6%) showed a Ki-67 of <2%, a few cases showed slightly higher proliferation indices.

Similar results have been observed in our study in which out of 35 patients 23 had Ki 67 index between 1-8 (benign 18, borderline 5), 6 patients had Ki 67 index between 9-16 (borderline 2, malignant 4) and 6 patient also had Ki 67 index >16 (borderline 2, malignant 4), Histopathologically benign tumor had Ki 67 index < 8. ($p< 0.000$). Not even a single patient of malignant phylloides tumor had Ki 67 index < 8($p< 0.000$). Sensitivity and specificity for group including both borderline malignant and malignant phylloides tumor was 70.58% and 100%. While the sensitivity and specificity to diagnose malignant phylloides tumor alone was 100%. All the subject of our study were divided into two groups, Ki 67 index less than 8 was labeled as benign and Ki 67 index more than 8 labeled as malignant, taking this presumption, the diagnostic accuracy of test for combined borderline malignant and malignant tumor was 88.46% and for malignant phylloides tumor alone was 100%. PPV and NPV of test for combined borderline malignant and malignant tumor and for malignant phylloides tumor alone was 100%. No significant difference was observed in the expression of Ki 67 in relation to longest diameter of tumor ($p >0.05$). Fisher exact `t` test did not showed significant difference ($p >0.05$) in between CD10 and Ki 67 for diagnosing a malignant phylloides tumor however, in observation it is seen that Ki 67 had higher sensitivity and specificity as compared to CD 10 to diagnosed malignant phylloides tumor (100%, 100% vs. 87.5%, 88.88%).

Relationship observed between diameter of tumor and increasing malignant potential, as shown in study out of 18 patients of benign histology only two had size >10 cm, while out of 8 malignant cases 4 had size >10 cm and only one patient had size < 5 cm. However analytical study failed to prove this relation ($p >0.05$). No relationship was observed in our study between palpable lymph nodes and expression of Immunohistochemical marker CD 10 and Ki 67 ($p >0.05$). No metastasis was found in any of our patients. In 3 recurrent cases included in our study the CD 10 expression remained same in both primary and recurrent diseases, while Ki 67 expression increased in recurrent tumors as compared to their primary counterpart; however a sample size of two or three patients is too small to make a universally acceptable hypothesis and to formulate treatment guidelines.

Conclusion

There is no significant difference between mean age of benign, borderline and malignant phylloides tumors. We investigated CD 10 and Ki 67 and found a significant increase in expression of Stromal cells CD 10 and Ki 67. Ki 67 labeling index of ≤8 suggest benign diseases, a value of ≥8 is strongly suggestive of malignant Phylloides tumor. Expression of CD 10 and Ki 67 is increases in malignant tumor and a higher expression even in benign tumor need a close follow-up for these patients. Tumor size had significant relation with CD 10 expression, as size increases CD 10 expression also increases, but this relation was not observed for Ki 67. Other clinical parameter like lymphadenopathy or locally advanced disease has no such association. Distant metastasis is rare, but local recurrence can occur uncommonly. Tumor size had no significant association with histopahological type, recurrence or lymphadenopathy. Recurrence had no relation with malignancy but associated to surgical management, so excision with adequate margin (>1 cm) has recommended. Lymph nodes dissection is unnecessary if there is no metastasis to lymph nodes, because it has its own complication.

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