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Immunohistochemical Expression of C-Kit in Fibroepithelial Tumors of Breast

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Abstract: Fibroadenomas are the most common breast tumors. Phyllodes tumors are a fibroepithelial tumor composed of an epithelial and a cellular stromal component. They may be considered benign, borderline, or malignant depending on histologic features. CD117, also called KIT or C-kit receptor, is a proto-oncogene that its expression or mutations can lead to cancer. C-kit shows stromal expression in malignant phyllodes tumors. The present study included thirty fibroepithelial breast tumor cases. This work aimed to study the expression of C-kit in fibroepithelial tumors of the breast and its relation with their clinicopathological parameters. According to our results, there was a progressive increase in C-kit expression from benign to malignant tumors, all cases of fibroadenomas were negative for C-kit [except for one case showed weak staining (score 1)]. All cases of phyllodes tumor showed positive staining with variable degrees of intensity. The difference in results of the immunostaining between fibroadenomas and phyllodes tumors were statistically significant (P=0.001).Within the phyllodes tumor cases score1 immunostaining was seen in 80 % of the benign cases, only one benign phyllodes tumor case showed moderate staining score 2. The malignant cases showed moderate immunostaining (score 2) in 40 % of the cases and strong staining (score 3) pattern in 60 % of the malignant cases. The borderline cases showed score 2 in 60 % of cases and score 3 in 40 % of these cases. In conclusion, the notable increase in C-kit expression in the mammary fibroepithelial tumors provides strong evidence that C-kit receptor mediated tyrosine kinase involvement in the pathogenesis of phyllodes tumors and the therapeutic agent, tyrosine inhibitor (Glivec) may be a potentially useful drug for management or preventing their recurrence.

Key words: Fibroepithelial · Tumors · Breast · CD 117

INTRODUCTION

Fibroadenomas are the most common breast tumors in adolescent women. They also occur in a small number of post-menopausal women. Their incidence declines with increasing age and they generally appear before the age of thirty years, probably partly as a result of estrogenic hormonal fluctuation [1]. Phyllodes tumors (PT) are a fibroepithelial tumor composed of an epithelial and a cellular stromal component, with the stromal proliferation becomes autonomous and no longer requires a mitogenic stimulus from the epithelium, resulting in stromal overgrowth [2]. They may be considered benign, borderline, or malignant depending on histologic features including stromal cellularity, infiltration at the tumor's edge and mitotic activity. All forms of phyllodes tumors are considered breast cancer, as even the benign form is regarded as having malignant potential [3]. Nonetheless, accurate classification of phyllodes tumor continues to be challenging on some occasions, partly because PTs are morphologically heterogeneous, with different areas of the same tumor showing different appearance, including areas morphologically indistinguishable from FA that may lead to diagnostic under-interpretation especially on examining limited material as fine needle aspiration or tru-cut needle biopsies [4]. CD117, also called KIT or C-kit receptor, is a cytokine receptor expressed on the surface of hematopoietic stem cells as well as other cell types. This receptor binds to stem cell factor (a substance that causes certain types of cells to grow). Altered forms of this receptor may be associated with some types of cancers [5]. Mutations of c-kit gene can cause activation

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of tyrosine kinase function of the C-kit protein, leading to ligand- independent tyrosine kinase activity and uncontrolled cell proliferation by downstream signaling. By such mechanism c-kit is implicated in the tumorigenesis of a variety of neoplasms [6]. The original rationale for studying C-kit expression in phyllodes tumor arose out of its similarities with gastro intestinal tumor (GIST), Both share certain criteria's such as spindled, CD34-positive stromal cells and show a spectrum of behavior from benign to malignant. However, the investigations that have looked at *C-kit* expression in phyllodes tumors have had varying results [7]. Accurate assessment of the level of expression of *C-kit* and further elucidation of the role it may play in the tumorigenesis of phyllodes tumors is an essential first step in determining the potential effectiveness of tyrosine inhibitors as therapeutic agents of phyllodes tumors [6].

This work aimed to study the expression of C-kit in fibroepithelial tumors of the breast and their correlation with tumor clinicopathological parameters.

MATERIALS AND METHODS

The study included thirty cases of fibroepithelial breast tumor in female patients who underwent lumpectomy or mastectomy whether simple or modified radical mastectomy. They were retrospectively obtained as paraffin blocks from Kasr El-Eini Pathology Department and private pathology laboratories from January 2011 to December 2011. Serial sections were cut 4 μm in thickness and stained by:

- Hematoxylin and Eosin (H&E) to revise the diagnosis, type and grading according to mitotic count into benign, borderline and malignant [8] of the tumor.
- Sections were obtained from each case and mounted on super frost TM slides for immunohistochemical analysis. Sections were deparaffinized and brought to water. Antigen retrieval was performed using citrate buffer pH 6 in water bath. Immunohistochemical staining was performed using an automated immunostainer (Dako Cytomation Autostainer S3400). The primary antibody used was Dako Cytomation rabbit anti human C-kit polyclonal antibody (Dako Cytomation, code A4502) used at a dilution range of 1:400 to 1:600 when applied on formalin-fixed, paraffin-embedded sections of fibroadenomas and phyllode tumors of the breast and using 20 minutes heat-induced epitope retrieval in target retrieval solution (code S1700) and 30 minutes incubation at room temperature with the primary antibody. Binding and detection were performed using Dako Cytomation En Vision TM peroxides dual link kit (K5007). According to Tsutsui *et al.* [9], immunoreactivity to C-kit is classified as positive (membranous/cytoplasmic) and negative reaction. Whereas for more accurate results, further reclassification according to variable intensities, into 0= negatively stained, 1= mild intensity, 2 = moderate intensity and 3 = marked (strong) intensity were done. A paraffin section of GIST was stained with C-kit and used as a positive control.

Statistical Analysis: Statistical analysis was performed using Fisher exact test. A relation considered statistically significant at $P < 0.05$.

RESULTS

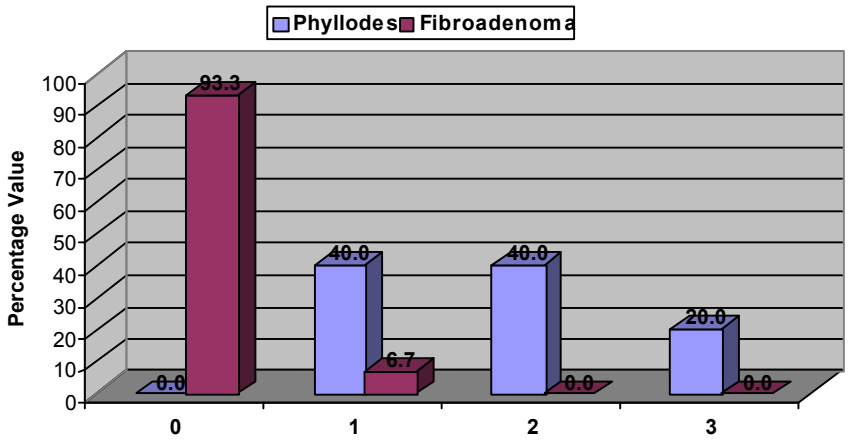
The age of the cases ranged from 15 up to 40 years in both phyllodes tumor (15 cases) and fibroadenomas (15 cases), distributed in the different age. The peak of age incidence for fibroadenomas lies in the age group (25-30 y), while the peak in the phyllodes tumor was in the age group (35-40 y), however the association of each of them with the corresponding age group was statistically insignificant ($P = 0.059$). All fibroadenoma cases showed rubbery grayish white cut section with slits. They ranged in size from 3 to 6.5 cm in diameter. Most of these cases (66.7%) were more than 5cm in diameter. Cases of phyllodes tumor showed rubbery leaf-like cut section and ranged in size from 3.5 to18 cm in diameter. Most of the cases as well were above 5 cm in diameter. Thus, the size above 5cm in diameter was not correlated to the diagnosis ($P = 0.682$). In 53.3% of fibroadenoma cases the tumor was bilateral, while all cases of the phyllodes tumor was unilateral and the difference was statistically significant

Table 1: Immune staining score of C-Kit in the studied cases

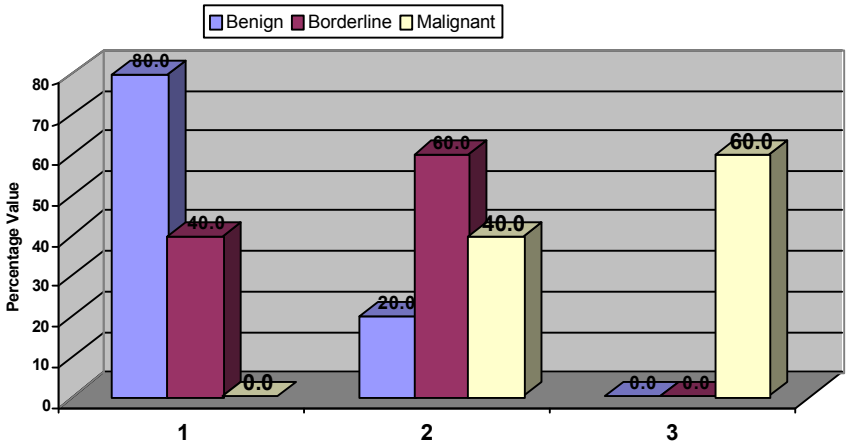
	Type			
	Fibroadenoma		Fibroadenoma	
Immune score	No	%	No	%
Score 0	14	93.3	0	0.0
Score 1	1	6.7	6	40.0
Score 2	0	0.0	6	40.0
Score 3	0	0.0	3	20.0
Total	15	100.0	15	100.0

Table 2: Immunoscoring of C-kit in the different grades of phyllodes tumor in the studied cases

	Grade					
	Benign		Borderline		Malignant	
Immune score	No	%	No	%	No	%
Score 1	4	80.0	2	40.0	0	0.0
Score 2	1	20.0	3	60.0	2	40.0
Score 3	0	0.0	0	0.0	3	60.0
Total	5	100.0	5	100.0	5	100.0



Graph 1: Demonstrating the results of immunostaining score of C-kit stain in the studied cases.



Graph 2: Demonstrating immunoscoring of C-Kit in different grades of phyllodes tumor.

(P<0.002). Cases of phyllodes tumor were graded according to mitotic count into benign, borderline and malignant. Hemorrhage was present in two cases of the phylloids tumor (13.3%). All cases of fibroadenoma were negative for C-kit, except for one cases showed weak staining (score 1) (Fig.2), on the other hand cases of phyllodes tumor showed positive staining with varying degrees of intensity, 40.0% showed score1 staining profile, 40.0% showed score 2 staining profile and 20% showed score 3 staining. The difference in results of immunostaining between fibroadenomas and phyllodes tumors was statistically significant (P =0.001) (Table 1 and Graph 1). There is statistically significant difference regarding relation between age and immune score as P value<0.05 (P = 0.016). The highest percentage of those with score 0 detected among those within age group (20-35 years), while the highest percentage of those with score 1 and 2 was detected within age groups (30-35 years).There is no statistically significant difference regarding relation between size and immune score as

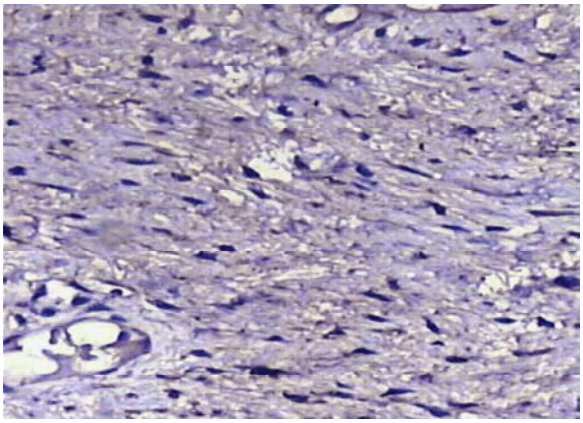


Fig. 1: Fibroadenoma with negative cytoplasmic staining to C-Kit in stromal cells (score 0) (C-kit, X 200).

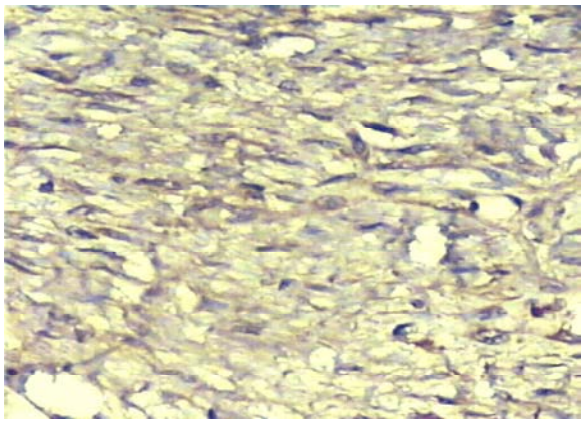


Fig. 2: Benign phyllodes tumor, mild cytoplasmic staining to C-kit in stromal cells (score 1) (C- kit, X 200).

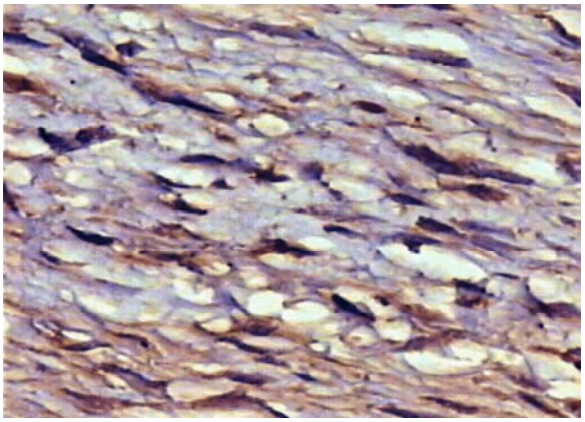


Fig. 3: Malignant phyllodes tumor with strong cytoplasmic staining to C-kit in stromal cells (score 3) (C- kit, X 400).

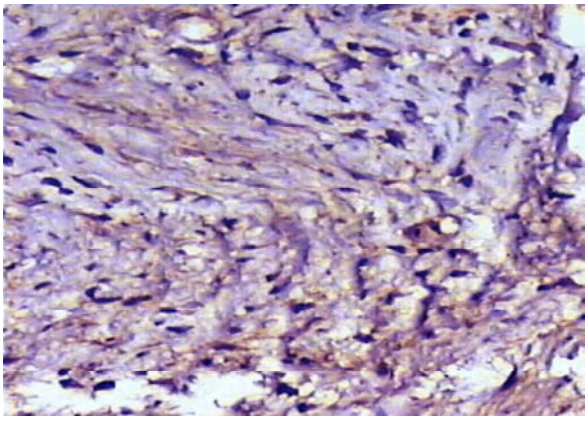


Fig. 4: Borderline phyllodes tumor, moderate cytoplasmic staining to C-kit in stromal cells (score 2) (C- kit, X 200)

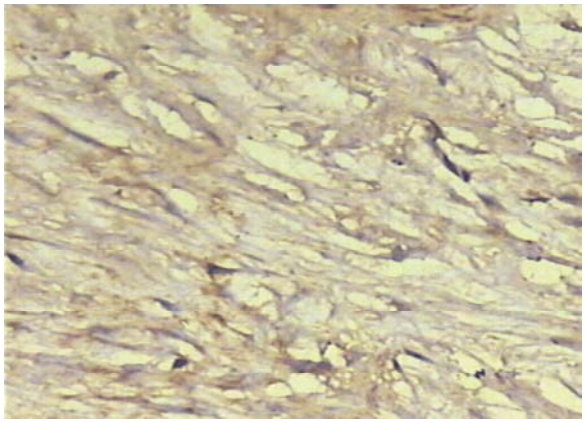


Fig. 5: Borderline phyllodes tumor, mild cytoplasmic staining to C-kit in stromal cells (score 1) (C- kit, X 200).

P value>0.05 (P = 0.117). Score1 immunostaining was seen in 4 cases (80%) of benign cases and only one case of benign phyllodes tumor case showed moderate intensity staining (score 2) (Fig. 3), on the other hand in the malignant cases showed moderate intensity (score 2) in 2 cases (40%) and showed strong intensity (score 3) in 3 cases (60%) of the studied cases (Fig. 4). The borderline cases showed score 2 in 60% of cases and score 1 in 40% of the studied cases (Fig. 4 and 5). The association between malignancy and high scores (2 and 3) is significant (P = 0.027). There is no statistically significant difference regarding relation between immunostaining score and the spread as P value > 0.05 (P= 0.100).

DISCUSSION

Fibroadenomas are the most common breast tumors in adolescent women. They also occur in a small number of post-menopausal women. Their incidence declines with increasing age and they generally appear before the age

of thirty years, probably partly as a result of estrogenic hormonal fluctuation. Although fibroadenoma is considered a neoplasm, some authors believe fibroadenoma arises from hyperplasia of normal breast lobule components [1]. Phyllodes tumors are a fibroepithelial tumor composed of an epithelial and a cellular stromal component. They may be considered benign, borderline, or malignant depending on histologic features including stromal cellularity, infiltration at the tumor's edge and mitotic activity. All forms of phyllodes tumors are considered breast cancer, as even the benign form is regarded as having malignant potential [3]. According to the current study, the age of the cases ranged from 15 years up to 40 years in both phyllodes tumor and fibroadenomas. The peak of age incidence for fibroadenoma lies in the (25-30 yrs) age group with the mean26.8, while the peak of the phyllodes tumor was in the (35- 40 yrs) age group and the mean 32.1, however the association of each of them with the corresponding age group was statistically insignificant (P = 0.059). The presented age incidence in the study was not conclusive being not a cohort study and due to the small size of the studied cases. Tse *et al.* [10] studied a total of 214 fibroepithelial tumors, 33 of which were fibroadenomas with age ranged from 20 to 58 years and 181 cases of phyllodes tumor ranging in age from 14 to 77 years. They noticed that there was a progressive significant increase in the age from fibroadenoma to phyllodes tumor of benign, borderline, malignancy and frank malignancy. Kleer *et al.* [11] studied phyllodes tumor cases only, the age of their study cases ranged from 18 to 86 and they noticed that phyllodes tumor rarely occur before the age of 35. On the other hand, Kuijper *et al.* [12] studied fibroadenoma with the mean age for their study cases was 33.4+12.1 SD. As well, Olu-Eddo and Ugiagbe [13] studied fibroadenoma in larger number in patients and found that the mean age was 20-29 years. Tan *et al.* [7] stated that although the age of presentation in both fibroadenomas and phyllodes tumors may give some diagnostic clue, however it should not be overemphasized as a differential point. In the available Egyptian registries, only the malignant phyllodes tumor cases are encountered, with no available details about age at presentation, tumors' size and other biologic features.

The gross pathological features of fibroadenoma cases were having rubbery grayish white cut section with slits. They ranged in size from 3cm up to 6.5cm in diameter. Most of the cases (66.7%) were more than 5cm in diameter. Cases of phyllodes tumor showed rubbery leaf- like cut section and ranged in size from 3.5cm to18cm. Most of the cases as well were above 5cm in diameter. Thus, the above 5 cm diameter size was not correlated to the diagnosis (P = 0.682). Also, in several studies found that the wide range in size in both groups and the absence of definite cut off value were observed by Tan *et al.* [7]. In this study, 53.3% of fibroadenoma cases the tumor was bilateral, while all cases of the phyllodes tumor was unilateral and the difference was statistically significant (P=0.002). The presence of bilateral fibroadenomas was noticed by Tan *et al.* [7], accounting for 39% of their study cases keeping with our results, Bilateral phyllodes was a very rare finding in all studies and presented as case reported by Ezeome *et al.* [14] and another case reported by Cohn-Cedermark [15] and Trabelsi *et al.* [16].

In the current study, the presence of hemorrhage was noticed in two cases of the phyllodes tumor. Sheen-Chen *et al.* [17] recorded the presence of hemorrhage in phyllodes tumors and referred this to the patient use of progesterone supplement for menstrual induction. Tse *et al.* [10] and Wurdinger *et al.* [18] found that hemorrhage may be present but not a very common feature that may be related to the rapid increase in size and rapid rate of growth. In our study, all cases of fibroadenoma were negative for C-kit, except for one case showed weak staining (score 1), on the other hand cases of phyllodes tumor showed positive staining with varying degrees of intensity, 40.0% showed score 1 staining profile, 40.0% showed score 2 and 20% showed score 3 staining. The difference in results of immunostaining between fibroadenomas and phyllodes tumors was statistically significant (P =0.001).Within the phyllodes tumor cases Score1 immunostainng was seen in 80% of benign cases, only one benign phyllodes tumor case showed moderate staining score 2. On the other hand, all the malignant cases showed moderate (score 2) in 40% of cases and strong (score 3) pattern in 60% of cases. The borderline cases showed score 2 in 60% of cases and score3 in 40% of cases. The association between malignancy and high scores (2 and 3) is statistically significant (P = 0.027). Thus, there was a notable progressive increase in C- kit expression from benign to malignant tumors. In contrast of our results, Djordjevic and Hanna [6] assumed that the apparent C-kit expression in the stromal component of phyllodes tumors is not substantiated by a molecular finding of any activating mutations and is likely a mast cell phenomenon. Similarly, Niezabitowski *et al.* [19] supported this finding. Both studies found that C-kit is an unlikely player in the pathogenesis of fibroepithelial lesions of the breast, it has neither a diagnostic nor a prognostic role in phyllodes tumors. As well, by studying

17 cases of PTs by immunohistochemical stain for c-kit and studying 14 of them by Kit mutation analysis by sequencing technique Bose *et al.* [20] found no role of C-kit mutation in the pathogenesis of the PTs. On the other hand, Esposito *et al.* [21] confirmed the expression of C-kit by the stromal cells in the fibroepithelial tumors in correlation to the tumor grade and found that this marker could be used as adjunctive marker of malignancy in these tumors.

Similarly, Ilić *et al.* [22] described 35 cases of mammary phyllodes tumor (20 benign, 6 borderline and 9 malignant) and found that intracytoplasmic C-kit expression was associated with a pathological diagnosis of malignancy, correlating with increasing grade ($p<0.05$). As well, Jara-Lazaro and Tan [2] noticed the similar progressive increase and proposed that c-kit may be a possible contributor to stromal proliferation in fibroepithelial tumors, presumably participating in the process of cell cycle progression. In conclusion, we join Tse *et al.* [10] the conclusion that the notable increase in C-kit expression in the mammary fibroepithelial tumors, provides strong evidence that C-kit receptor mediated tyrosine kinase involvement in the pathogenesis of phyllodes tumors and the therapeutic agent, tyrosine inhibitor (Glivec) may be a potentially useful drug for their management or preventing their recurrence.

CONCLUSION

From the results of the current study we concluded that CD117 plays a role in the pathogenesis of breast fibroepithelial tumors, with its expression by the stromal cells is correlated to the tumor grade, with no obvious correlation with other clinic pathologic parameter including the age of the patient, the size of the tumor or the presence or absence of the spread. Thus, we recommend the use of the CD117 as adjunctive marker of malignancy in these tumors and suggest that the therapeutic agent, tyrosine inhibitor (Glivec) may be a potentially useful drug for its management or preventing their recurrence.

Conflicts of Interest: There are no conflicts of interest.

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