C-Kit (CD-117) Expression in Malignant Phyllodes Tumors of Breast.

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ABSTRACT

Background: Malignant Phyllodes tumor (MPT) is a fibro epithelial neoplasm of breast, and has an age adjusted incidence rate of 3.1 per one million women. The recurrence rate of MPT reported by various studies varies from 356%, and 27% according to WHO, usually detected within 3 years. .

Objective: The objective of this study is to, "Determine the frequency and grading of c-kit (CD-117) immunohistochemical (IHC) expression in 'malignant Phyllodes tumors' of breast in patients presenting to tertiary care hospital"

Materials & Methods: This was a descriptive, cross sectional study, conducted from 1st July 2010 to 31st December 2010 in Histopathology, Pathology and Microbiology departments of, Aga Khan University Hospital (AKUH), Karachi Forty-three consecutive cases of biopsy proven malignant Phyllodes tumor of breast were stained using ckit antibody by immunohistochemistry. Grading of ckit immunohistochemical expression was assessed, in neoplastic stromal cells.

Results: Out of these 53.5% (23) were grades 1, 23.3% (10) were grade 2 and 23.3% (10) were grade 3. Further categorizing these, 46.5 % (20) were positive and 53.5% (23) were negative for ckit.

Conclusion: Results of our study showed that 46.55 % of cases of malignant Phyllodes tumor showed positive staining for ckit. Twenty three% of our cases showed strong staining for ckit and thus are the most likely candidates for targeted treatment against this gene by drugs such as Imatinib mesylate.

Key Words: *Malignant Phyllodes tumor, Immunohistochemistry, ckit, chemotherapy, treatment, Imitanib mesylate, Gleevac.*

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INTRODUCTION

Malignant Phyllodes tumor (MPT) is a fibro epithelial neoplasm of breast, and has an age adjusted incidence rate of 3.1 per one million women[1-3]. The recurrence rate of MPT reported by various studies varies from 356%, and 27% according to WHO, usually detected within 3 years. [¨] Metastatic rates in MPT range from 12% to 65% predominantly involving the lungs, followed by pleura, bones and rarely liver and central nervous system[4-7]. The development of distant metastasis is uniformly fatal. Treatment is mainly surgical, wide local excision with clear margins or mastectomy.[8-11] Role of adjuvant chemotherapy and radiotherapy is not well established.[12-16]

CKIT (CD117) is a proto-oncogene that encodes for a trans-membrane tyrosine kinase receptor (TKR) protein (CD117), expressed in hematopoietic stem cells, mast cells and basal cells of skin, melanocytes, germ cell and interstitial cells of Cajal[17-20]. The kinase receptor is activated by its ligand stem cell factor, produced by interstitial cells. Activation leads to receptor dimerization and activation of downstream signaling cascades. Oncogenic KIT mutations cause ligand independent activation of the TKR, driving tumor genesis. These mutations can be detected at molecular level using RT-PCR and FISH and result in over expression of proteins at cellular level which can be detected by Immunohistochemistry (IHC) using anti ckit (CD117) antibody on paraffin embedded fixed tissue[22,23].

Imatinib Mesylate (IM, Gleevec / Glivec) is a 2-phenylaminopyridine compound that selectively inhibits TKR, preventing phosphorylation and cellular proliferation[24,25]. C-kit mutations play an important role in the development of gastro-intestinal 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 Imatinib may be successful mode of treatment in cases of MPT with ckit overexpression[27].

The objective of this study is to, "Determine the frequency and grading of c-kit (CD-117) immunohistochemical (IHC) expression in 'malignant Phyllodes tumors' of breast in patients presenting to tertiary care hospital".

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MATERIAL AND METHODS

This descriptive, cross sectional study was conducted at Section of Histopathology, department of Pathology and Microbiology, Aga Khan University Hospital (AKUH), Karachi for a period of six months.

Based on the estimated frequency of 50% a sample size of 43 was calculated bound to the error of estimation of 15% or more and a confidence interval of 95%

$n = z^2_{1-\alpha/2} P (1-P)/A^2$

The source of data included 43 consecutive cases of biopsy proven MPT, received in the department, during the study period. These specimens were grossed according to the guidelines. Formalin fixed, paraffin embedded tissue sections were stained with Haematoxylin and Eosin (H&E) The morphology was studied microscopically and diagnosis was established by a resident and a consultant. Malignant Phyllodes tumor was diagnosed in breast lesions exhibiting three or more of the following features; Increased stromal cellularity, marked stromal cell nuclear atypia, mitotic count more than 10/10HPF (high power fields), stromal overgrowth and infiltrative margins.

Informed consent was taken from the patients whose specimens were utilized for the study. The biopsy proven cases of MPT were stained for ckit antibody using immunohistochemistry by qualified medical technologists. The authors assessed grading of ckit IHC expression. Data was collected on a pre-designed proforma; variables including age, histological diagnosis, ckit grading and positive/ negative staining was recorded.

IMMUNOHISTOCHEMICAL STAINING FOR CKIT:

A section with the largest number of tumor cells covering an area of at least 1x1cm^2 (measured microscopically) was selected for each case of MPT and stained with the antibody c-kit (DAKO, Denmark) on automated immunostainer, using 20 minutes heat induced epitope retrieval in target retrieval solution. After washings and blockage of endogenous peroxidase, the slides were incubated with the primary antibody for c-kit for 30 minutes, followed by detection with a streptavidin-biotin immuno-enzymatic antigen system. Positive and negative controls were included with each batch.

GRADING OF CKIT IMMUNOHISTOCHEMICAL EXPRESSION

The brown cytoplasmic staining of the neoplastic stromal tumor cells was considered as positive for c-kit expression and the expression of staining was graded as; Grade 0; 0% of neoplastic stromal cells positive, Grade1; 1% to 25% of neoplastic stromal cells positive, Grade3; 26% to 50% of neoplastic stromal cells positive and Grade 3; 51% or more neoplastic stromal cells positive. Samples with grades 0 and 1 were considered negative. Samples with grades 2 and 3 were considered positive.

DATA ANALYSIS

Statistical analysis was done using SPSS version 16 and proportions (percentages) were reported for categorical variables like c-kit expression, while means with standard deviation were reported for continuous variable, i.e. age of the patient and size of the tumor. Stratification was done for age and grading of the c-kit expression to assess the impact on outcome.

RESULTS

A total of forty-three cases of malignant Phyllodes tumor were included in the study. Mean age for MPT was 39 years, which is a decade younger than the age in most studies (50-60 years). [Table 1] All of these were stained for c-kit. Out of these cases, 23 (53.5 %) had 25% of stromal cells positive and were graded 1. Ten (23.3 %) had staining in the range of 26-50% and were graded as 2. Ten (23.3%) cases of MPT showed a strong staining for c-kit antibody, i.e. more than 50% of cells and were graded as 3. [Figure 2] None of the cases were scored as grade 0. Samples with grades 0 and 1 were considered negative and those with grades 2 and 3 were considered positive. Thus, twenty-three cases (53.5%) were categorized as negative, where as twenty cases (46.5%) were positive. [Table2]

DISCUSSION

Phyllodes tumors are fibro epithelial tumors of breast, arising from stroma of breast and are classified as benign, borderline and malignant on the basis of morphology[1]. MPT is a rare neoplasm that constitutes 0.3-0.5% of all breast tumors.[1] Incidence rate peaks in the 45-49 year age group in western studies; however in our population it is reported at a younger age which is in keeping with the average age of 40 years in our study.[7] MPT exhibits marked stromal cellularity, marked stromal cell nuclear atypia, a mitotic count of ten or more per ten high power fields, stromal overgrowth and infiltrative margins. [Figure1]

Given the advent of specific therapy targeted at ckit tyrosine kinase receptor, the known propensity of mammary Phyllodes tumors to recur or metastasize and preliminary results suggesting increased c-kit expression in malignant phyllodes tumor, it is imperative to follow on this observation in our population.

In Pakistan many studies have been published regarding Phyllodes tumor including case series and case reports.[9] One of the study shows expression of CD 10 in Malignant Phyllodes tumor and correlates it with metastasis and recurrence.
Most of the studies, conducted internationally, have used all three categories of PT and have compared the IHC staining of ckit in benign, borderline and malignant tumors, however, owing to sample size problems and budget restrictions, we selected only malignant Phyllodes tumor. The earliest study published showed ckit expression in 75 % of MPTs. Carviho et.al, showed ckit expression in 100% of MPT with preferential staining in subepithelial stroma. Our study, however, showed diffuse stromal staining in positive cases. The largest study with a sample size of 335 cases of PT showed positivity in 77% of MPT. Two studies showed ckit expression in 46 & 50 % of MPT,[7] these results are close to our study, which shows positivity in 48% of MPT. Molecular techniques were also used in some of the above-mentioned studies. Two studies found point mutations of unknown significance in exons 10 & 11. Another study found that none of the activating mutations described in GIST in exons 9, 11, 13 &17 of the ckit gene were found in their cases of important, suggesting that MPT must use other growth factors to sustain their growth. CKit expression has been reported using IHC on tissue microarrays, correlating it with poor prognosis and decreased survival tumor grade. Although no mutations in ckit gene were identified, but protein expression was reported to be associated stromal cellularity, atypia, mitotic activity and invasive margins. C-Kit overexpression in the stroma of MPT directly correlates with kit gene copy number increases identified via genomic Polymerase chain reaction (PCR)

Trials are being carried out to determine the efficacy of Imatinib either alone or in combination with other chemotherapeutic agents in a number of tumors where ckit expression is common such as small cell lung carcinoma, malignant melanoma and germ cell tumors[10]. Such trials can be carried for MPT and metastatic MPT. Twenty three percent of our cases showed strong staining for ckit. Although molecular studies were not done, these cases are the most likely candidates for ckit mutation and thus susceptible for targeted therapy against this mutation.

CONCLUSION

Results of our study showed that 46.55 % of cases of malignant Phyllodes tumor showed positive staining for ckit. Twenty three% of our cases showed strong staining for ckit and thus are the most likely candidates for targeted treatment against this gene by drugs such as Imatinib mesylate.

Table no. 1. Age and size of Important

	Number	Min.	Max.	Mean	Standard deviation
Age in years.	43	17 years	77 year	39.44	13.890
Size in centimeters.	43	2 cm	30 cm	12.15 cm	6.377

Table no. 2. Grading of staining of MPT

IHC grading of c-kit expression.	Percentage of neoplastic stromal cells positive.	Frequency of grading.	Percentage %	Groups of ckit expression	Frequency	Percentage %
0	0%	0	0 %	Negative	23	53.5%
1	1-25%	23	53.5 %			
2	26-50%	10	23.3 %	Positive	20	46.5%
3	>50%	10	23.3 %			
Total		43	100 %	43	43	100%

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