Research Article

C-kit expression in breast carcinoma – A study of 62 cases of breast carcinoma

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Abstract

C-kit, also known as KIT or CD117, is a protooncogene that encodes a transmembrane tyrosine kinase receptor, which is a type 3 transmembrane receptor for MGF (mast cell growth factor, also known as stem cell factor). C-kit plays various important roles in hematopoiesis, melanogenesis, spermatogenesis and the development of interstitial cells of Cajal. C-kit was first identified as the cellular homolog of the feline sarcoma viral oncogene v-kit. Mutations in this gene are associated with various types of malignant tumours. C-kit expression was shown to be reduced in breast carcinomas whereas normal epithelium showed an almost 100% expression. The present study aims at evaluating the c-kit expression in breast cancer, to correlate with clinicopathological factors such as age, menstrual status, associated risk factors, stage, nuclear grade, Nottinghams prognostic index, estrogen receptor, progesterone receptor and Her2neu status and to evaluate its prognostic value. A total number of Sixty two cases of invasive ductal carcinomas were immunostained for c-kit protein. C-kit positivity was noted in varying number of cases. This was correlated with staging, ER and PR status and Her 2 neu expression.

Keywords: C-kit, breast cancer, ER, PR, Her2 neu.

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INTRODUCTION

C-kit, also known as KIT or CD117, is a protooncogene that encodes a transmembrane tyrosine kinase receptor, which is a type 3 transmembrane receptor for MGF (mast cell growth factor, also known as stem cell factor). C-kit plays various important roles in hematopoiesis, melanogenesis, spermatogenesis and the development of interstitial cells of Cajal. C-kit was first identified as the cellular homologue of the feline sarcoma viral oncogene v-kit. Mutations in this gene are associated with various types of malignant tumours. C-kit expression was shown to be reduced in breast carcinomas whereas normal epithelium showed an almost 100% expression. The present study aims at evaluating the c-kit expression in breast cancer, to correlate with clinicopathological factors such as age, menstrual status, associated risk factors, stage, nuclear grade, Nottinghams prognostic index, estrogen receptor, progesterone receptor and Her2neu status and to evaluate its prognostic value. A total number of 62 cases of invasive ductal carcinomas were immunostained for c-kit protein. C-kit positivity was noted in varying number of cases. This was correlated with staging, ER and PR status and Her 2 neu expression.

MATERIALS AND METHODS

Case selection

A total of 62 cases of carcinoma of breast was selected prospectively among the patients admitted in Sri Ramachandra Medical College And Research Institute, Porur from April 2008 to November 2010. All cases of operable carcinoma breast were included for the study. Cases of recurrent carcinoma breast and in situ carcinoma were excluded from the study. All cases underwent thorough clinical examination, staging and fine needle aspiration cytology and/or trucut biopsy to confirm the diagnosis of malignancy. Specific attention to risk factors like early menarche, late menopause, nulliparity, family history of breast carcinoma and obesity were given. All the patients were treated surgically. Resected specimens were fixed in 10% neutral buffered formalin within 3

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hours. Overnight fixation, followed by gross examination, lymph node dissection and tissue sampling was performed as per standard protocol. In addition to routine hematoxylin and eosin staining, immunohistochemical stains were performed for ER, PR and Her2neu on paraffin wax embedded blocks. The tumour was graded by Nottingham histological scoring system. Pathological staging of the disease was done. Final combined pathological and clinical staging was performed based on AlCC 7th edition, 2010. ER, PR and Her 2 neu stains were interpreted by the standard guide lines. The criterion given in table 1 is used for the molecular classification of breast carcinoma.

Table 1: Molecular classification of Breast carcinoma by immunohistochemistry

	ER	PR	Her2 neu
Luminal A	Positive	Positive	Negative
Luminal B	Positive	Positive	Positive
Her2	Negative	Negative	Positive
Triple Negative	Negative	Negative	Negative

Immunostaining was done for C-kit and its expression was evaluated in normal, in situ and invasive component of the lesions (paraffin blocks were selected representing normal, in situ and invasive component wherever applicable). C-kit expression was graded as 0, 1, 2 or 3+ based on the intensity of staining and the percentage of the cells as in Table 2.

Table 2: Grading of C-KIT expression (USCAP guidelines)

C-kit score	Intensity of staining	% of cells	Interpretation
0	None	None	Negative
+	Mild	>10%	Positive
++	Moderate	> 10%	Positive
+++	Marked	Any %	Positive

The c-kit expression was compared with age, risk factors, premenopausal/postmenopausal status, and lymph node status, histopathology of tumour, grade of tumour, Nottinghams prognostic Index, ER, PR and Her 2 neu and statistically analysed.

RESULTS

Total number of cases included in the study was 62. All the cases showed c-kit positivity (100%) in normal epithelium. 32 out of the 62 cases (52%) expressed c-kit positivity in the tumour cells and 30 cases (48%) showed loss of ckit expression. 61 % (11/18) patients in the pre-menopausal group expressed c-kit whereas only 45 % (20/44) patients in the post-menopausal group expressed c-kit. This was not found to be statistically significant. A total of 51 patients were above 40 years, and 26 (51%) were positive for c-kit and 25(49%) were negative for c-kit. 11 patients were below 40 years of age, and 6(54.5%) were positive for c-kit and 4 (45.5%) were negative for c-kit.

Table 3: Age and ckit expression

Age	C-kit +ve	C-kit –ve
Age >40	26 (51 %)	25 (49%)
Age <40	6 (54.5%)	5 (45.5%)

Table 4: Risk factors and ckit expression

Risk Factor	C-kit +ve	C-kit –ve
Late menopause	7 (64%)	4 (34%)
Benign breast lump excision	1 (9%)	2 (16.5%)
Nulliparity	3 (27%)	2(16.5%)
Family history of breast cancer	0	2 (16.5%)
Family history of other cancer (carcinoma stomach, carcinoma endometrium)	0	2(16.5%)

Table 5: Size of tumour and ckit expression

Size of tumour	C-kit +ve	C-kit –ve
T1	3 (9%)	4 (13%)
T2	17 (53%)	18 (60%)
T3	12 (38%)	6 (20%)
T4	0	2 (7%)

Forty seven patients had invasive ductal carcinoma out of which 23 (49%) were positive for c-kit and 24 (51%) were negative. The only invasive lobular carcinoma in our study was negative for c-kit expression. 14 patients were of other subtypes out of which 9 (4 mucinous, 2 medullary, 2 papillary and 1 squamous) were positive for c-kit and 5 (3 apocrine and 2 mucinous) were negative for c-kit.

Table 6: Histologic type and ckit expression

Histopathology	C-kit +ve	C-kit –ve
Invasive ductal	23 (49%)	24 (51%)
Invasive lobular	0	1 (100%)
Others	9 (64%)	5 (36%)

Of 8 patients with grade I tumour, 3 (36%) were c-kit positive and 5 (64%) were negative. 39 patients had grade II tumour, 22 (57%) were c-kit positive and17 (43%) were c-kit negative. 7 (46%) of 15 patients with grade III tumours were positive for c-kit and 8 (54%) were negative.

Table 7: Histologic grade and ckit expression

Grade of tumour	C-kit +ve	C-kit -ve
Grade I	3 (36%)	5 (34%)
Grade II	22 (57%)	17(43%)
Grade III	7 (46%)	8 (54%)

Table 8: Nottinghams prognostic index (NPI) and ckit expression

NPI	C-kit +ve	C-kit –ve
NPI <3.4	11 (50%)	11 (50%)
NPI 3.4-5.4	14 (50%)	14 (50%)
NPI >5.4	7 (58%)	5 (42%)

Table 9: Clinical stage and ckit expression

	C-KIT positive	C-KIT negative
Stage I	2 (50%)	2 (50%)
Stage II	16 (48%)	17 (52%)
Stage III	13 (54%)	11 (46%)
Stage IV	1 (100%)	0

Table 10: ER, PR and Her2neu and ckit expression

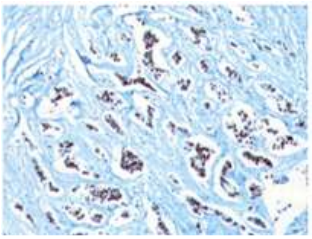
Hormonal status		C-KIT positive	C-KIT negative
Estrogen receptor	ER Positive	13 (40%)	19 (60%)
	ER Negative	19 (63%)	11 (37%)
Progesterone receptor	PR Positive	12 (46%)	14 (54%)
	PR Negative	20 (55%)	16 (45%)
Her2 neu	Positive	13 (46%)	15 (54%)
	Intermediate	3 (43%)	4 (57%)
	Negative	16 (59%)	11 (41%)

Correlation of ER, PR and Her 2 neu with c-kit showed no significant relationship.

Table 11: Molecular subtype and ckit expression

	C-KIT positive	C-KIT negative	Total
LUMINAL	7 (41%)	10 (59%)	17
HER 2 /neu	15 (44%)	19 (56%)	34
TRIPLE NEGATIVE	10 (91%)	1 (9%)	11

In this study we had 11 cases that were triple negative. All 11 patients had either a grade II or III tumours. 10 of these 11 patients were positive for C-kit expression. Eight out of the 11 triple negative and c-kit positive tumours were invasive ductal carcinoma, 2 were medullary and 1 papillary type. 1 papillary carcinoma was triple negative and c-kit negative.



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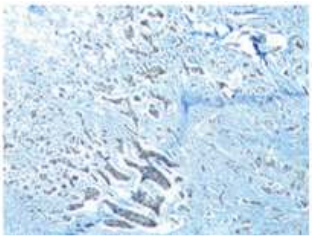

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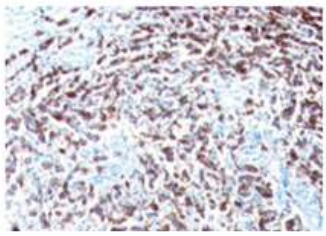

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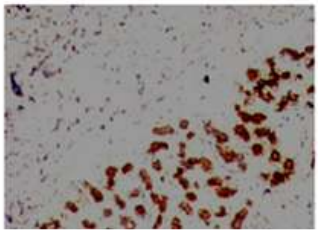

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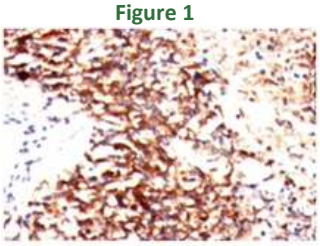

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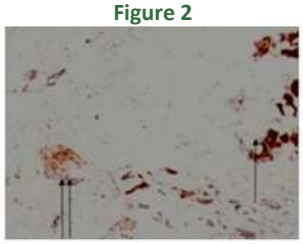

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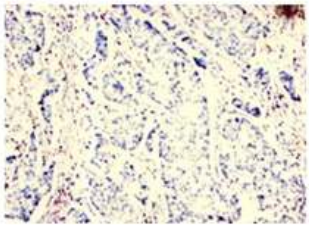

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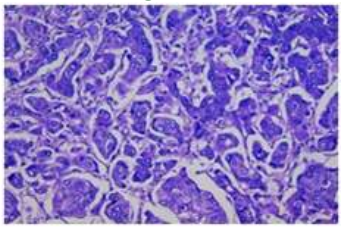
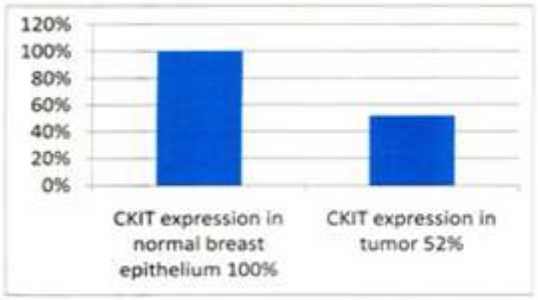
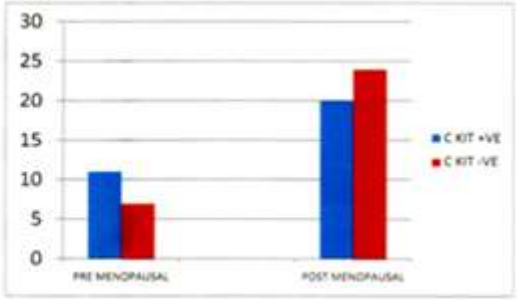


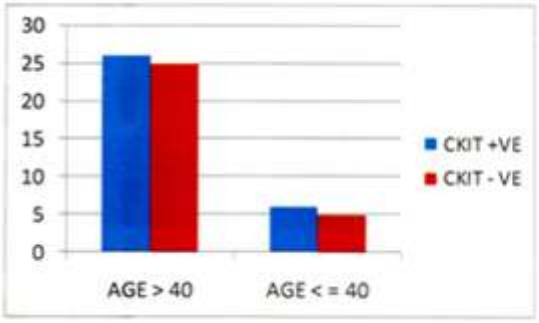
Figure 8

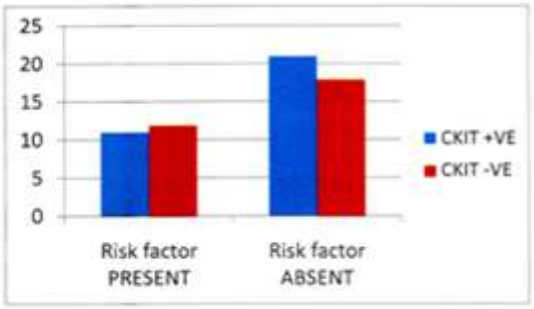
Legend:

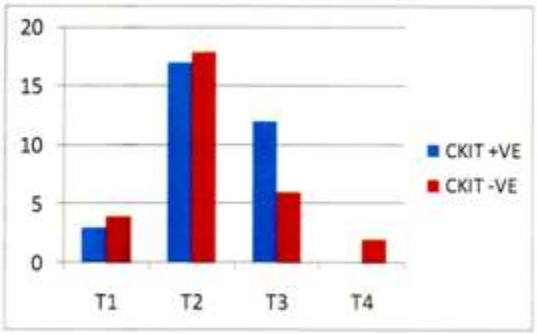
Figure 1: PR positivity in nucleus of tumour cells (x100)
Figure 2: ER positivity in nucleus of tumour cells (x100)
Figure 3: Her2 neu positivity (3+) in nucleus of tumour cells (x100)
Figure 4: IHC – Normal breast tissue showing 3+ intensity for c-kit (x100)
Figure 5: IHC – Tumour cells showing c-kit positivity (3+) (x100)
Figure 6: IHC – Showing normal breast tissue showing 3+ positivity for c-kit (single arrow) and 1+ positivity for tumour cells (double arrow) (x100)
Figure 7: IHC – c-kit negativity in tumour cells (x100)
Figure 8: H and E Breast cancer Nottingham Grade 3 (x200)

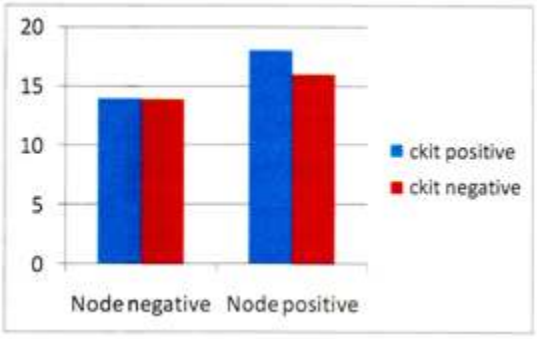


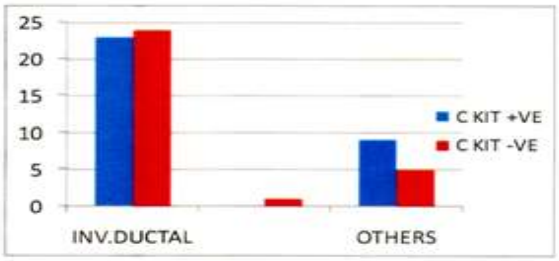


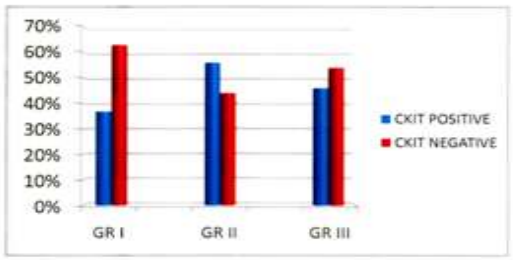


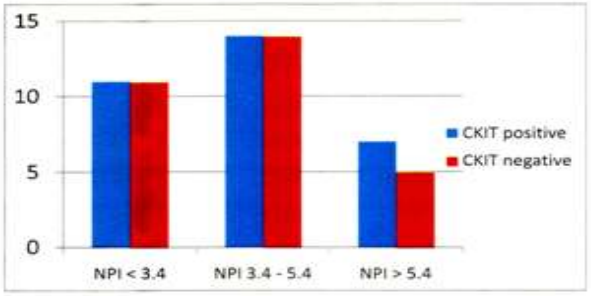


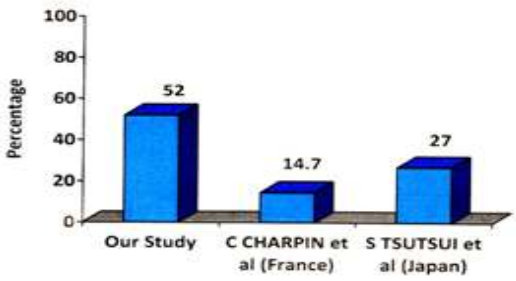












DISCUSSION

Breast cancer is a heterogeneous disease encompassing a number of distinct biological entities that are associated with specific morphological and immunohistochemical features and clinical behaviour. Despite this morphological heterogeneity however, patients can practically be classified into three main groups for management and therapy according to

- Hormone receptor positivity (ER/PR)
- Presence of Her 2 neu (c-erb2) amplification
- Absence of these two characteristics (Triple negative cancers)

The latter group of patients lack specific targets for hormone and Trastuzumab therapy and there is a need to identify new targets for tailored treatment. This study is done to evaluate the c-kit status in breast carcinoma and the possible use of anti-c-kit therapy for breast cancer. Ckit is a 145 kDa transmembrane glycoprotein, a member of the receptor tyrosine kinase subclass III family that includes receptors for platelet derived growth factors, macrophage colony stimulating factor, and flt-3 ligand. C-kit is expressed in a variety of normal cells and tissues and plays an important role in normal physiological processes such as hematopoiesis, embryogenesis, and melanogenesis. In breast cancer the

expression of c-kit is known to vary from 1% to 95%. In breast cancer, ckit expression correlates with higher tumour grade and is detected in 80% of metastatic breast cancer. In the present study, c-kit expression was present in all the normal breast epithelium. The expression in carcinoma in situ was observed in 11 out of 19 (58%) patients. Carcinoma in situ was not present in 43 patients. Out of 62 carcinomas, 32 (52%) were found to express c-kit in breast carcinoma. These findings indicate a gradual reductionin ckit expression from benign to insitu to invasive carcinomas. The c-kit expression in invasive breast carcinoma in the present study was high when compared to studies done by C Charpin *et al* and S Tsutsui *et al*. The c-kit expression was found to be reduced in carcinoma in situ and invasive carcinoma when compared to the c-kit expression in normal breast. The c-kit positivity in patients with lymph node metastasis was slightly higher than those who did not have lymph node metastasis. But this was not statistically significant (P value - 0.510). In a study done by S TSUTUSI *et al*, the correlation was significant between lymph node metastasis and c-kit. The variation observed could be explained by the limited number of cases in this study.

Table 12: Lymph node metastasis and ckit expression

	Lymph node metastasis	Number of patients	c-kit positive	c-kit negative	P value
S TSUTSUI *et al*, Japan	Absent	117	45(38%)	72(62%)	<0.0001
	Present	99	14 (14%)	85 (86%)	
Present study	Absent	28	14 (50%)	14 (50%)	0.510
	Present	34	18 (53%)	16 (47%)	

When analysing the histological type of breast carcinoma in c-kit positive patients, most of them (23/32, 71%) were of invasive ductal carcinoma. The rest were of medullary, mucinous, squamous and papillary type. There were no statistical significance when correlating ckit expression with the presence of risk factors and size of the tumour, nuclear grade, Nottingham's prognostic index, stage of the disease , ER, PR and Her2 neu status. This corresponds to the study done by Tsutsui *et al*. C-kit was expressed in 91% of triple negative cases and was statistically significant (P view studies also indicate the possible role for ckit in tumour progression through its interaction with stromal elements, although the exact mechanism is still unknown. In a study by Nielsen TO *et al*, there was a significant relationship between ckit expression and basal like breast cancer subtype, with the majority of ckit positive tumours belonging to the basal like breast cancer subtype. Women with basal like triple negative breast cancer tended to be younger, have a histologically high grade tumour, with positive nodal status, than those whose tumours were non basal-like. Possibilities exist for future targeted therapy for this challenging group of breast cancers. (Thike AA *et al*)

CONCLUSION

In conclusion, this study demonstrates over expression of ckit in normal breast epithelium and a reduced expression in in situ and invasive breast carcinomas. In addition, based on the analysed studies and the findings in this study, evaluation of C-kit may prove helpful as a prognostic marker. Ckit expression does not correlate with age of patient, menstrual status, associated risk factors, size of tumour, grade, lymph node status and Nottingham's prognostic index. In triple negative patients, as the c-kit expression is high, suggesting a definitive role in targeted therapy (such as Imatinib mesylate). However a larger study population is required to evaluate for the same.

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