Original Research Article

C-KIT expression in breast carcinoma and its correlation with TNM stage, grade and expression of ER, PR, HER2/NEU& BRCA1

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Received: 12-06-2021 / Revised: 08-07-2021 / Accepted: 27-08-2021

Abstract

Aims and objectives: To evaluate the c-kit (CD117) expression and its prognostic valueinvasive breast carcinoma and also to correlate its expression with grade of the tumor, TNM staging, ER, PR, Her2/neu & BRCA1 immunomarkers. **Materials and methods:** Our study was conducted from July 2011 to December 2012 in a tertiary care hospital, during this period we received a total of 80 modified radical mastectomy specimens of which ER, PR, Her2/neu and BRCA1 were done is 29 cases. Clicinopathological factors were evaluated in detail for these cases, C-Kit expression was analyzed by immunohischemistry method using Quick's score and correlated with grade of the tumor, TNM staging, ER, PR, Her2/neu & BRCA1 markers. **Results:** Highest incidence of breast carcinoma was noted in 30 to 40 years of age group and 68.9% were below 50 years of age. Majority of the tumors were of high grade and of T2 stage, accounting to 72.5 % and 65.5% respectively, Lymph node metastasis was noted majorly in N2 category (41.3 %) and none showed distant metastasis. On molecular classification, majority of the tumors were of luminal A type followed by triple negative of 41.4% and 20.6 % respectively. 24.2 % of all the cases expressed C-kit among invasive breast carcinoma of no special type, higher grade, high tumor size, Luminal A and triple negative cases of molecular classification. Lack of C-kit expression was observed majorly in less than 50 years of age group, less tumor size &lymph node metastasis, Luminal B and HER2/Neu positive types on molecular classification. Statistical correlation was attained between C- Kit expression and ER, PR negativity and Her2/Neu positivity. **Conclusion:** C-Kit (CD117) expression needs to be assessed routinely for all invasive breast carcinoma, either negative or positive will have its prognostic and therapeutic implications respectively.

Keywords: C-Kit expression,Breast carcinoma, TNM stage, tumor, Luminal A type

Introduction

Breast cancer is a multifaceted disease comprising of distinct biological subtypes with diverse natural history, presenting a varied spectrum of clinical, pathologic and molecular features with different prognostic and therapeutic implications[1]. Breast carcinoma usually occurs in pre- and post-menopausal women with slight increase in incidence in younger population. Familial cases tend to occur at an early age. The assessment of ER along with PR status of breast carcinoma gives a stronger predictive power to predict the outcome of endocrine therapy[2]. Amplification and over-expression of the HER-2/neu gene and protein have been identified in 10%-34% of invasive breast cancers, which show an adverse prognosis in either node-negative or node-positive disease (independent prognostic marker). The c-kit (CD117) proto- oncogene, located on chromosome 4q11-12, encodes a transmembrane tyrosine kinase receptor, the phosphorylation of tyrosine residues is the prerequisite for activation of a variety of signal transduction pathways involved in proliferation, apoptosis, and tumorigenesis. C-kit protein expression has been found in a wide variety of malignant tumors including myeloid leukemia, small cell carcinoma of lung, seminomas, and gastrointestinal stromal tumors, but is frequently diminished in other tumors, in. Several studies have shown that c- kit is highly expressed in normal breast epithelium but is present at only low levels or is completely lost in primary invasive breast cancer or breast cancer metastases[3]. With this background, we aimed to evaluate the c-kit expression and its prognostic value in breast carcinoma in our region and correlate its expression with grade, expression of other independent markers like ER/PR, and HER-2/neu using IHC, which determines the protein expression levels cost effectively.

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Aims and objectives

1. To evaluate the c-kit (CD117) expression in invasive breast carcinoma.
2. To correlate with grade of the tumor, TNM staging and ER, PR, Her2/neu& BRCA1 immunomarkers.
3. To evaluate the prognostic value of c-kit expression in invasive breast carcinoma.

Materials and methods

This study was conducted in the Department of Pathology, in co-operation with departments of General surgery and Radiotherapy at Government general hospital/ Rangaraya Medical College (RMC), Kakinada, from July 2011 to December 2012.

We received a total of 80 cases of modified radical mastectomy specimens. Only those cases for which immunohistochemistry was done in our institute were included in the study. Tumor size was assessed during the grossing of the specimen after sufficient fixation. Appropriate tumor tissue and all the lymph nodes identified were processed.Immunohistochemistry was used for determining C-kit expression, ER/PR status, HER2/neu expression and BRCA1 expression. The C-kit expression was correlated with clinico-

pathological parameters, ER/PR status, HER2/neu and BRCA1 expression of the tumors was studied.

The analysis for the expression of C-kit, ER/PR receptors, HER2/neu& BRCA1 was done by using the respective antibody as per the standard procedure. The scoring of the stained sections was done by two independent observers & the average value taken as the expression of protein.IHC stained slides were evaluated according to the Quick score which takes proportion Scoring was of cells stained and intensity of staining into consideration. The staining was evaluated on the invasive component only. Well preserved and well stained areas of the sections were assessed done by two independent observers and the average value was taken as the protein expression.

Nuclear staining was assessed for ER& PR

1. A score for the proportion of stained cells, 0 = no nuclear staining, 1 = < 1% nuclear staining, 2 = 1-10% nuclear staining, 3 = 11-33% nuclear staining, 4 = 34-66% nuclear staining and 5 = 67-100% nuclear staining.
2. Intensity of staining (0 = no staining, 1 = weak staining, 2 = moderate staining, 3 = strong staining) were assigned to each tumor.

The score for the proportion of cells stained and the score for the intensity of staining were added to get the total score, which ranged from 0-8.

HER-2/neu expression was assessed according to CAP guidelines

- Tumors that show strong circumferential staining (referred to as 3+ staining) in > 30% of cells by IHC
- Tumors that show moderately strong circumferential membrane staining (referred to as 2+ staining)
- Tumors that show little or no protein expression by IHC (referred to as 0 or 1+ staining)

BRCA1protein expression was assessed based on

Positive staining of normal breast epithelial cells that either coexisted on the tumor sections and or normal breast tissue sections from the same breast was used as a control. The protein expression levels in tumor sections were measured in three discontinuous classes:

1. When the immunoreactivity was comparable to that of the normal breast epithelium or nuclear staining was observed in > 50% of tumor cells, it was classified as class 3 i.e., wild type or normal expression.
2. When the staining was clearly weaker than normal surrounding cells or nuclear staining occurred in 20% to 50% of tumor cells, it was classified as class 2 i.e., reduced expression.
3. When there was no staining or nuclear staining occurs in < 20% of tumor cells, it was classified as class 1, that is absent/ markedly reduced expression.

C-KIT expression was assessed using Quick score

1. A score for the proportion of stained cells (0= no staining, 1= 1-20%cytoplasmic/membranous staining, 2= 21-50% cytoplasmic/ membranous staining, 3 = 51-100% cytoplasmic/membranous staining. Intensity of staining (0= no staining, 1= weak staining, 2= moderately staining, 3= strong staining) were assigned to each tumor.
2. The score for the proportion of cells stained and the score for the intensity of staining were added to get the total score, which ranged from 0 = 6.

Results

In our study, highest incidence of breast carcinoma was noted in 30 to 40 years of age group and 68.9% were below 50 years of age. Majority of the tumors were of high grade(72.5 %) and of T2 stage of 2 cm - 5 cm size(65.5%), Lymph node metastasis was noted majorly in 4 to 9 lymph nodes group of N2 category (41.3 %) and none showed distant metastasis. On molecular classification, majority of the tumors were of luminal A type(41/4%) followed by triple negative(41/4%). 24.2 % of all the cases expressed C-kit among invasive breast carcinoma of no special type, higher grade, high tumor size, Luminal A and triple negative cases of molecular classification. Lack of C-kit expression was observed majorly in less than 50 years of age group, less tumor size, less no of lymph node metastasis, Luminal B and HER2/Neu positive types in molecular classification.

Table:1 Correlation of various clinicopathological &. prognostic parameters of invasive breast carcinoma with C-kit expression and its significance.

S. NO	Clininopathological&prognostic factors	Total cases n %	CKIT Expression		Significance
			Positive	Negative	
1	AGE				
	< 50 years	20 (68.9%)	4 (20%)	16 (80%)	No
	> 50 years	9 (31.1%)	3 (33.6%)	6 (66.4%)	
2	Tumor's size				
	T1(< 2 cm)	1(3.4%)	0(0%)	1(100%)	
	T2(2-5cm)	19(65.5%)	4(21.1%)	15(78.9%)	No
	T3(>5cm)	9(31.1%)	3(33.4%)	6(66.4%)	
3	Lymph node status				
	No	9(31.1%)	2(22.3%)	7(77.7%)	
	N1	7(24.2%)	2(28.5%)	5(71.5%)	No
	N2	12(41.3%)	3(25%)	9(75%)	
	N3	1(3.4%)	0(0%)	1(100%)	
4	Distance metastasis				
	Mx	0	_	_	
	M0	0	_	_	No
	M1	0	_	_	
5	Tumor grading				
	G1	1(3.4%)	0%	1(100%)	
	G2	7(24.1%)	1(14.3%)	6(85.7%)	No
	G3	21(72.5%)	6(23.5%)	15(76.5%)	
6	Tumor staging				
	Stage I	1(3.4%)	0%	1(100%)	
	Stage IIA	5(17.3%)	3(60%)	2(40%)	No
	Stage IIB	8(27.5%)	1(12.5%)	7(87.5%)	
	Stage IIIA	15(51.7%)	3(20%)	12(80%)	

7	Histological classification Invasive ductal carcinoma Invasive Lobular carcinoma Atypical Medullary carcinoma	 27(93.2%) 1(3.4%) 1(3.4%)	 6(22.2%) 0% 1(100%)	 21(77.8%) 1(100%) 0%	 No
8	ER Expression Positive Negative	 13(49.8%) 16(55.2%)	 4(30%) 3(18.7%)	 9(70%) 13(81.3%)	 No Yes
9	PR Expression Positive Negative	 13(49.8%) 16(55.2%)	 4(30%) 3(18.7%)	 9(70%) 13(81.3%)	 No Yes
10	Her2/neu Expression Positive Negative	 7(24.1%) 22(75.9%)	 1(14.2%) 5(22.7%)	 6(85.8%) 17(77.3%)	 Yes
11	Molecular subtypes Luminal A Luminal B Her2/neu positive Triple negative	 12(41.4%) 3(10.3%) 4(13.7%) 6(20.6%)	 4(33.3%) 0 0 3(50%)	 8(66.7%) 3(100%) 4(100%) 3(50%)	 No
12	BRCA Expression Present/ normal Absent/reduced	 16(55.2%) 13(44.8%)	 4(25%) 3(23%)	 12(75%) 10(77%)	 No

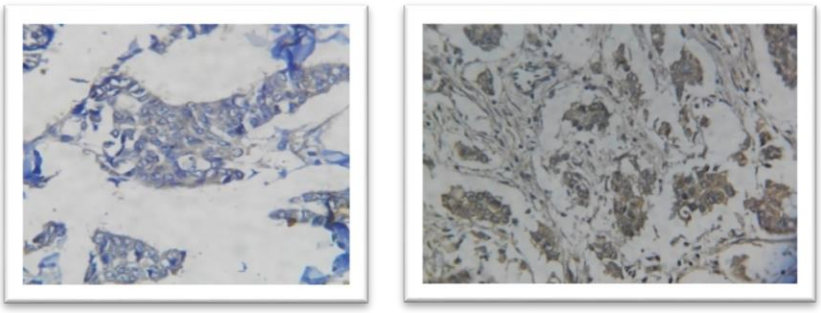

Fig 1: Tumor cells showing ckit positive expression

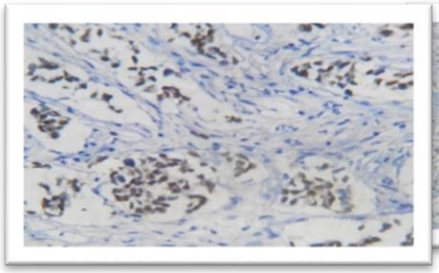

Fig 2:Tumor cells are er positive

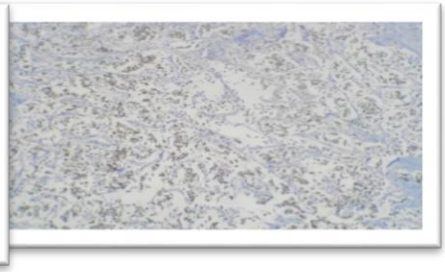

Fig 3: Tumors cells are pr positive

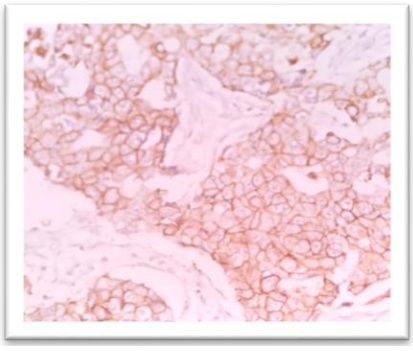

Fig 4: Tumor cells are her2/neu positive.

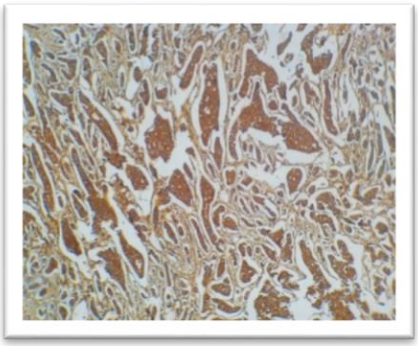

Fig 5:Brca1 normal expression in breast tumor cells

Discussion

Breast cancer is a heterogeneous disease. The C-kit expression is present in both normal epithelium and malignant cells of breast. In our study the expression of C-kit immunomarker is slightly lower than the other published data, the immunoreactivity being 24.2% of the cases. The proliferation and differentiation of normal breast cells are regulated by C-kit signaling pathway[4] and loss of C-kit expression during progression from normal breast to malignant breast is described by Lennartss on et al[5]. In this present study C-kit expression in invasive breast carcinomas were correlated with clinicopathological parameters and prognostic immunohistochemical markers such as ER, PR, Her2/neu and also BRCA1 by immunohistochemistry.

In our study the youngest age presented with invasive carcinoma was 30 years, oldest age was 75years. This was compared with the study done by Carreno et al[6], Honma et al[7], Micello et al[8]. In India there is change in trend towards younger age group in recent years. In the present study majority of breast cancers were observed below 50 years of age and the age group being 30- 40 years accounting to 41.4%.

Among the histological types, Invasive ductal carcinoma NOS type is the most common type which is 93% in our study, this was in concordance with Albrektsen et al[9], Shirley SE et al[10] and AM Dauda et al study[11]. The other variants in our study were Atypical medullary carcinoma and invasive lobular carcinoma.

Majority of the breast carcinomas were in T2 stage in our present study, which is similar to the studies done by Lakshmi et al[12] and Christine L. Carter, Carol et al[13]. The proportion of T2 tumors are more in Indian population when compared to western population.

In our study Grade II were more common than the other grades which coincides with Carey et al[14], GG Vanden Eynden et al[15] and Qiuj, Yang et al[16] studies and 41% of cases showed lymph node metastasis with 4-9 positive lymph nodes category which is N2.

On molecular classification, based on ER, PR, Her2/neu, luminal A category were the maximum number of cases, which is in concordance with Adedayo et al[17] study.

On immunohistochemistry, C-kit expression in our study was 24.2% of invasive breast carcinomas, which was in concordance with S. Tsutsui et al[18], Hitoshi Tsuda et al[19] and Maha M Amin et al[20] studies. While C-kit expression among various other studies were Abdallah et al[21] of 90.5%, susruthan et al of 52%, Palmu et al [23] of 82.6%, Tahany M Shams et al of 75% and Hitashi Tsuda et al[19] of 10%.

On comparison of C-kit expression with clinicopathological parameters like age, histological type, tumor size, grade and lymph node metastasis, no significant statistical correlation was seen in our present study. In our study majority of the cases were invasive carcinoma of NOS type of which 22% expressed C-kit, 100% of Atypical medullary variant expressed C-kit, but not invasive lobular carcinoma variant. Abdallah et al[21] study showed 90.5% positive expression of c-kit in IDC of NOS type, 100% positivity in special variants, while Susruthan et al[22] showed 49% positivity in IDC NOS, 69% positivity in other subtypes and Hitoshi Tsuda et al[19] study showed 10% positivity in IDC NOS type.

In our study positive C-kit expression in tumor grade 1,2,3 tumors is 0%, 14.3% and 28.5% respectively while negative C-kit expression in tumor grading was observed in 100%, 85.7% and 76.5% respectively. There was no statistical significance observed, which was in concordance with Hitoshi Tsuda et al study, while Tahany M Shams et al, Abdallah et al,Susruthan et alshowed statistical significance with higher grade and C-kit positive expression

Expression of C-kit in lymph nodeinvolvement, did not have any statistical significance but showed lack of C-kit expression in higher lymph node stage group of N3, in our study which was in concordance with MahaShomaf et al whereas Tsutsui et al[18] showed a significant relation between lymphnode metastasis and C-kit expression. This study 30% of ER, PR positive tumors expressed C-kit positivity whereas 81.3% of ER, PR negative tumors had lack of C-kit expression, which was statistically significant.

14.2% of Her2 positive cases showed C-kit positivity and 85.7% showed lack of C-kit expression which was statistically significant. which was in contrast with the studies done by Susruthan et al[22], Maha N Amin et al[20] showed strong association between C-kit positivity and Her2 positivity. With respect to BRCA1 expression, our study showed C-Kit expression in 23% (3/13) BRCA1 mutated cases (reduced or absent expression), similar study was done by Domagala et al[26] where they correlated BRCA1 association breast carcinomas with other IHC marker profile.

In our study, on correlating C-Kit expression with molecular classification of breast, luminal A group showed 33.3% of C-kit expression, luminal B showed 0% expression, Her2 positive category showed 10% C-kit expression and Basal like breast carcinomas showed 50% C-kit expression which was significant, even though statistical significance was not attained. Tahany M Shams et al[24] showed 75% of immunoreactivity for C-kit in triple negative basal like breast carcinomas. Nielson et al study and Susruthan et al showed high C-kit expression in triple negative breast cancers suggestive definitive role in targeted therapy[21-24]

Conclusion

C-kit expression is seen in 24.2% of primary breast cancer patients. Clinicopathological parameters like age of patient, size of tumor, grade of tumor and histological type did not show any significant correlation with C-kit expression. C-kit expression was seen more among invasive breast carcinoma of no special type, higher grade and size of tumor, Luminal A and triple negative categories of molecular breast classification. Lack of C-kit expression was observed majorly in less than 50 years of age group, less tumor size, less no of lymph node metastasis, Luminal B and HER2/Neu positive categories of molecular classification. C-kit was observed in 33% of luminal A and 50% of triple negative breast carcinomas, which suggests definitive role of targeted therapy.

CD117(C-Kit) expression needs to be assessed routinely for all invasive breast carcinoma, either negative or positive will have its prognostic and therapeutic implications respectively. However due to limitations of our present study being lower sample size, future studies with larger sample size are advised.

Acknowledgment

The authors would like to thank Department of Pathology for providing all the facilities to carry out this work.

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