Expression of stem cell growth factor receptor CD117 (C-Kit) in breast neoplasms

V. Manikanta[1], V. Siva Kota Reddy[2,*]

[1]Assistant Professor, Dept. of Pathology, Katuri Medical College & Hospital, Katuri Nagar, Guntur, Andhra Pradesh,
[2]Assistant Professor, Dept. of Pathology, Narayana Medical College & Hospital, Nellore, Andhra Pradesh, India

***Corresponding Author:**
Email: ktrdvs@live.com

Abstract

Aim: study designed to correlate the histopathological grading and IHC of Breast cancers and also to study the expression of stem cell growth factor receptor CD117(c-Kit) in stromal component, myoepithelial cells and ductal epithelial cells of non-neoplastic & neoplastic breast lesions.

Materials and Methods: A study of 50 women with breast neoplasm enrolled and their samples were examined by lumpectomy and mastectomy and fixed for immunohistochemistry with monoclonal rabbit anti-human c-Kit marker. 4-6 microns sections were stained with Haematoxylin and Eosin and grading was done according to modified bloom and richardson grading.

Results: In breast cancer, the expression of c-Kit observed to be high in malignant breast epithelium. Expression of c-Kit decreased in the benign ductal epithelium and infiltrating duct cell carcinoma. There is a significant increase in the expression of c-Kit in the stromal cells as the lesions progressed from benign to malignant phyllodes tumor. Reduction in c-Kit expression in malignant breast lesions may suggest its carcinogenic role in the breast.

Conclusion: There is decrease in the expression of c-Kit was observed between benign ductal epithelium (92%) and infiltrating duct cell carcinoma. There is a significant increase in c-Kit expression observed in the stromal cells as the lesions progressed between benign (16.5%) and malignant phyllodes (50%).

Keywords: CD117(c-Kit), Benign ductal epithelium, malignant breast epithelium, Haematoxylin and Eosin staining.

Introduction

Globally, approximately 1.6 million new breast cancer cases were diagnosed in 2010. Approximately 4, 25,000 women in the world died from this disease in 2010. In the past 25 years, incidence rates have risen about 30% in westernized countries. This increase may be due to changes in reproductive patterns and increase in the screening.[1]

The incidence of breast cancer in India is 22.9 per 100,000 one third that of western countries and the mortality rates are disproportionately higher. Breast cancer accounts for 22.2% of all new cancer diagnoses and 17.2% of all cancer deaths among women in India. Breast cancer in urban areas of India is three times higher than in rural parts of the country.[2,3]

A growing list of available antibodies, improved antigen retrieval techniques and a better understanding of biology have all contributed to the broader utility of IHC for solving diagnostic problems in breast pathology. Immunohistochemistry markers CD117(c-KIT) provide diagnostic, prognostic and therapeutic information.

Naturally, c-Kit (CD117) is a protooncogene which encodes a transmembrane tyrosine kinase growth factor receptor. Through interaction with its ligand, the C-Kit protein plays a role in hematopoiesis, melanogenesis and gametogenesis. c-Kit expression was observed in hematopoietic stem cells, tissue specific stem cells, tissue specific mast cells, germ cells, melanocytes and interstitial cells of Cajal etc. However c-Kit expression was not usually observed in normal squamous epithelium/glandular epithelium of the lung, endocervix, pancreas, prostate, stomach or the small and large intestines (Vanucchi MG et al., 1999).[4]

Over-expression of c-Kit has been found to affect proliferation in human neural, lung, colorectal, skin and prostate organs and leads to tumors (Sammarco I et al., 2004).[5]

In breast cancer, the expression of c-Kit represents a highly controversial subject, but the majority of studies have observed that the decreased c-Kit expression in malignant breast epithelium (Crisi GM et al., 2005).[6]

Previous study shows that c-Kit was highly expressed in benign lesions (Fibroadenoma, fibrocystic disease, sclerosing adenosis, tubular adenoma and benign phyllodes). c-Kit was highly expressed in myofibroblast/ fibroblast cells only in grade III ductal / lobular carcinomas. c-Kit was totally absent in stromal cells in benign lesions and in situ carcinomas, whereas c-Kit expression was weak in grade I and II carcinomas. In recent years, the role of c-Kit in the development of pre-invasive and invasive breast carcinomas has been investigated (Agatha Kondi-Pafiti et al., 2010).[7]

Hence the study was carried out to correlate IHC and histopathological grade of breast carcinomas and their CD117 expression.

Materials and Methods

A study of 50 women with Benign and Malignant lesions of the breast were included in the study. The study carried out at Department of Pathology, Dr. Pinnamaneni Institute of Medical Sciences and Research Foundation, Vijayawada. Samples were examined by lumpectomy and mastectomy and fixed

for immunohistochemistry staining with monoclonal rabbit anti-human CD117 marker. These sections stained with Haematoxylin and Eosin and grading was done according to Modified Bloom and Richardson grading.

Immunohistochemistry Procedure:

1. Deparaffinize, treat with xylene and ethanol, and wash with water.
2. Place slides in a probe tank conitaining antigen reterival buffer.
3. Probe tank is placed in the easy reterival oven at 96°C for 2 cycles (Each cycle 7-8 mintutes).
4. Prepare PBS solution (300 ml of sodium chloride + 204 ml of disodium hydrogen orthophosphate + 96 ml of sodium hydrogen orthophosphate, PH maintaining 7.2-7.4) When cooled, remove.
5. Treat with endogenous peroxidase block 3 drops (3% hydrogen peroxide solution, 10 minutes at room temperature and wash with PBS solution for 3 times.
6. Perform Power block 2 drops (10% normal goat serum-PBS, 10 minutes at room temperature) do not wash with PBS solutionand add primary antibody.
7. Place primary antibody-Kit and allow reacting for 30-40 minutes at room temperature, Wash with PBS for 3times.
8. Allow to react with Super enhancer solution 2 drops for 30 minutes at room temperature, and then wash with PBS solution.
9. Allow to react with SS labelled solution 3 drops for 30 minutes at room temperature and wash with PBS for 3 times.
10. Add DAB chromogen buffer solution 2 drops for 10 minutes at room temperature.
11. Wash with tap water, and then allow reacting with Meyer's hematoxylin for 10 seconds at room temperature.
12. Allow colour to develop in lukewarm water, and then dehydrate, clear, and mount.

Scoring systems used for CD117 (c-Kit) expression in breast neoplasms

	(Epithelial &Stromal cells)
Score 0:-	Negative (No staining)
Score 1+	Cytoplasm discretely and weakly moderately stained in 10% or more cells. (Weak positive)
Score 2+	Cytoplasmic staining Strong in >10% of cells (Strong positive)

Results

There are 32 cases diagnosed with Benign and 18 cases(36%) with malignant cancer. 11 cases in 21-30, 15 cases in 31-40, 11 cases in 41- 50, 2 cases in 51- 60 age group were enrolled. In 32 cases of benign tumors, 18 cases diagnosed with fibroadenoma, 6 cases of benign phyllods. In total of 18 cases of carcinoma, there are 16 cases diagnosed with infiltrating duct cell carcinoma, and 2 cases with malignant phyllods.

Table 1: CD117(c-Kit) expression in benign and malignant lesions

Type of lesions	CD117 negative	CD117 weak positive	CD117 strong positive
Fibroadenoma (18 cases)			
Ductal epithelial cells	1	4	13
Myoepithelial cells	18	0	0
Stromal cells	18	0	0
Tubular Adenoma (4 cases)			
Ductal epithelial cells	0	0	4
Myoepithelial cells	4	0	0
Stromal cells	4	0	0
Sclerosing Adenosis (4 cases)			
Ductal epithelial cells	0	2	2
Myoepithelial cells	4	0	0
Stromal cells	4	0	0
Benign Phyllodes(6 cases)			
Ductal epithelial cells	0	4	2
Myoepithelial cells	6	0	0
Stromal cells	5	1	0
Infiltrating duct carcinoma(16 cases)			

Ductal epithelial cells	16	0	0
Myoepithelial cells	16	0	0
Stromal cells	15	1	0
Malignant Phyllodes (2 cases)			
Ductal epithelial cells	2	0	0
Myoepithelial cells	2	0	0
Stromal cells	1	1	0

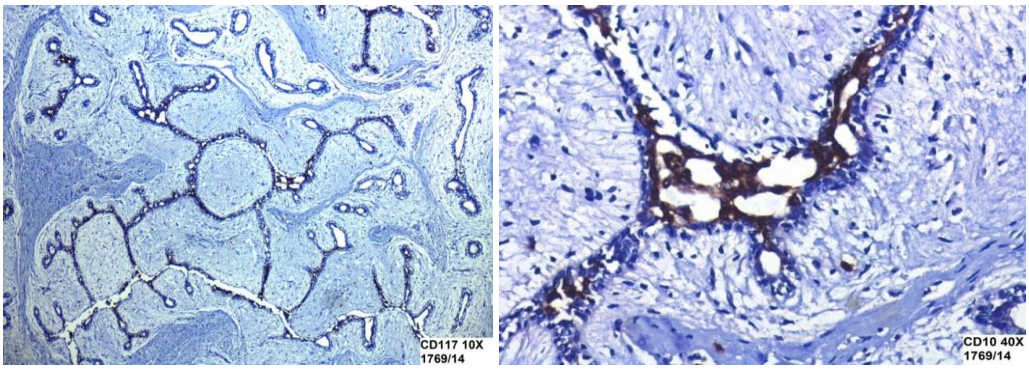

Fig. 1: Photomicrograph of fibroadenoma showing Strong positive cytoplasmic staining for c-Kit in the Ductal epithelial cells

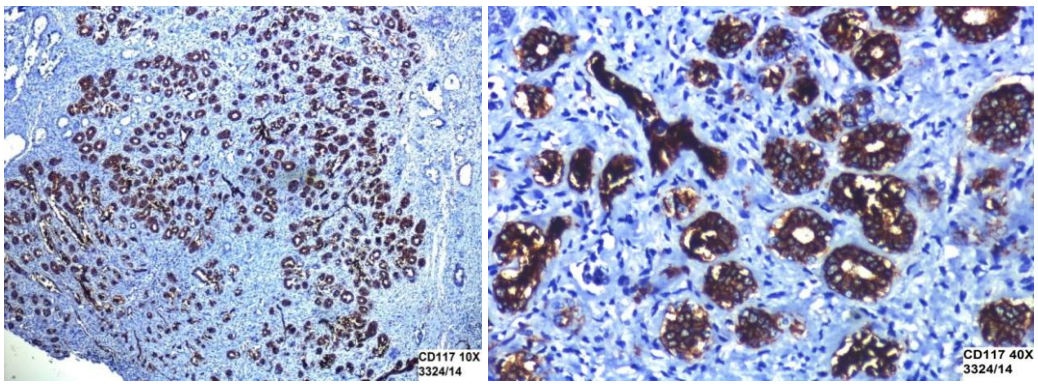

Fig. 2: Photomicrograph of tubular adenoma showing Strong positive cytoplasmic staining for c-Kit in the Ductal epithelial cells

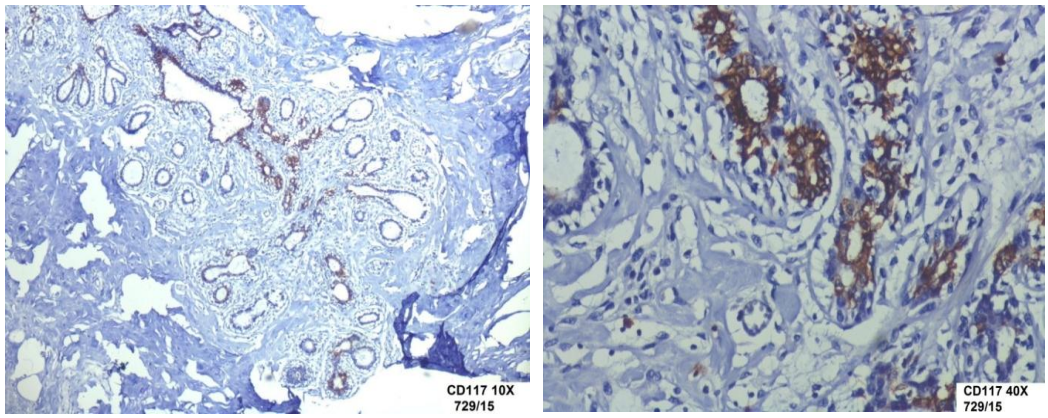

Fig. 3: Photomicrograph of sclerosing adenosis showing Strong positive cytoplasmic staining for c-Kit in the Ductal epithelial cells

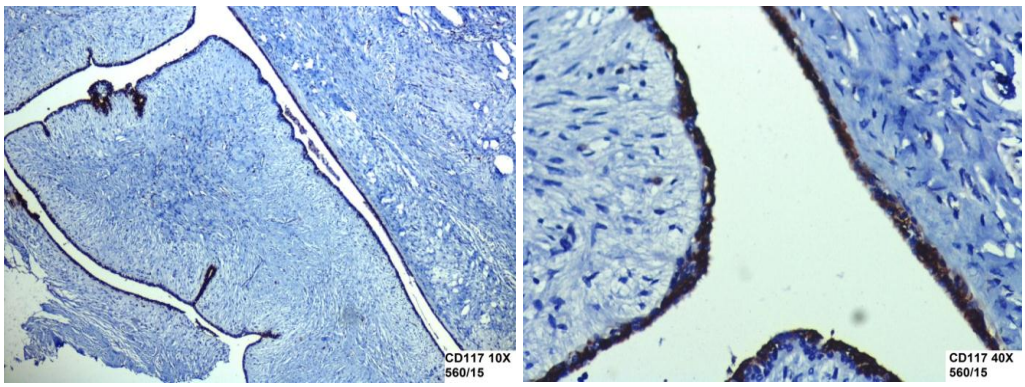

Fig. 4: Photomicrograph of benign phyllodes showing Strong positive cytoplasmic staining for c-Kit in the ductal epithelial cells

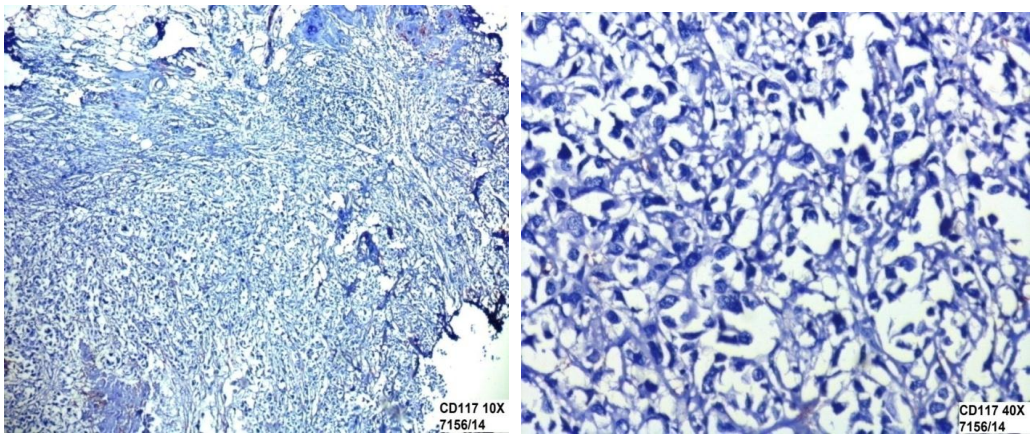

Fig. 5: Photomicrograph of infiltrating duct cell carcinoma showing Negative staining for c-Kit in the stromal cells

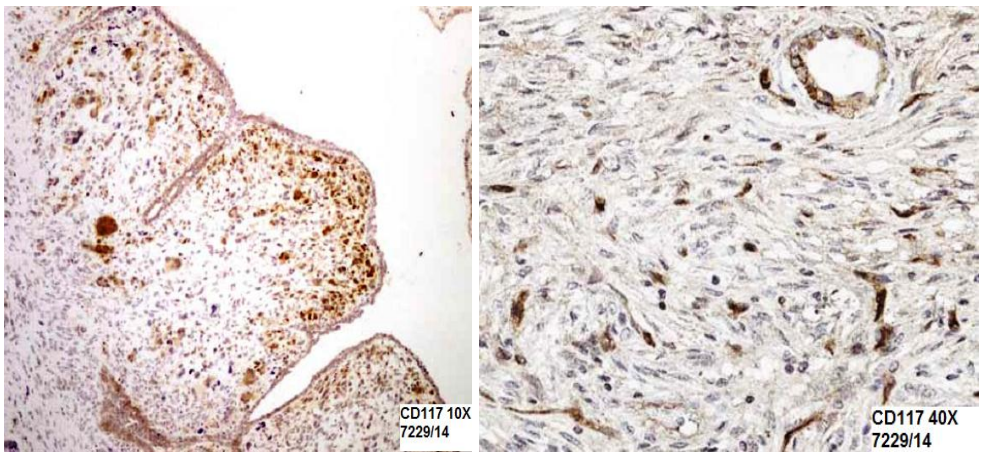

Fig. 6: Photomicrograph of malignant phyllodes weakly staining of stromal CD117 (c-Kit) expression

Discussion

50 samples immunohistochemistry was done with monoclonal rabbit anti-human CD117(c-Kit) antibodies during the period from August 2013 to August 2015. In the present study, majority (55%) of patients belongs to age group between 21-40 years. Whreas, 26% cases belongs to age group 41-50. This study shows similar concordance to age group of 42.9 years quoted by Maha M.Amin et al.[8]

64% cases diagnosed with beningn catogery, which is almost similar to the study conducted by Suzuko Moritani et al 2002, Agatha Kondi et al., 2010, Maha M. Amin et al., 2012, A N Kalof et al., 2015.[7-10]

In the present study infiltrating duct cell carcinoma predominated with 16 cases out of 18 cases (88.9%) and Malignant Phyllode show 2 cases out of 16 cases (11.1%).

Table 2: Comparison of CD117(c-Kit) Ductal epithelial cells expression in breast lesions

	Benign	Malignant
Ronald simon et al.,2004 [11]	82.5%	14.6%
Bojana D etal.,2008 [12]	82%	15.25%
Agatha Kondi et al.,2010 [7]	85.3%	13.75%
Maha M et al.,2012 [8]	87.5%	-
Present Study	92.5%	-

In the present study CD117(c-Kit) was consistently positive in Ductal epithelial cells of benign breast lesions. Comparison of CD117(c-Kit) Ductal epithelial cells expression was most commonly seen in benign breast lesions (92.5%) higher compared to the study conducted by Ronald Simon et al 2004, Bojana D et al 2008, Agatha Kondi et al 2010 and Maha M et al 2012.

Table 3: Comparison of CD117(c-Kit) Stromal expression in phyllodes

	Puay-Hoon Tan et al.,2005 [13]	Present study
Benign Phyllodes	3.39%	16.5%
Borderline Phyllodes	10.81%	-
Malignant Phyllodes	40%	50%

In the present study CD117(c-Kit) was consistently positive in Stromal cells of malignant phyllodes. There is a significant increase in CD10 expression in the stromal cells as the lesions progressed from benign to malignant phyllodes tumor, compared to the study conducted by Puay-Hoon Tan.[13]

Table 4: Comparison of CD117(c-Kit) stromal expression in malignant breast lesions

	Infiltrating duct cell carcinoma	Malignant phyllodes
Ronald simon et al.,2004 [11]	10%	50%
Bojana D etal.,2008 [12]	14%	44.5%
Agatha Kondi et al.,2010 [7]	18.5%	71.25%
Maha M et al.,2012 [8]	24%	56.75%
Present Study	-	50%

In the present study CD117 (c-Kit) was negative in stromal cells of infiltrating duct cell carcinoma and in malignant phyllodes (50%). Stromal expression of CD117(c-Kit) malignant phyllodes is similar (50%), compared to study conducted by Ronald simon, Bojana D, Agatha Kondi and Maha M.[7,11,12]

Conclusion

In Breast cancer, the expression of c-Kit found decreased in malignant breast epithelium. There is decrease in the expression of c-Kit from benign ductal epithelium to infiltrating duct cell carcinoma. There is a significant increase in c-Kit expression observed in the stromal cells as the lesions progressed from benign to malignant phyllodes tumor. Hence, reduction in c-Kit expression in malignant breast lesions may suggest its carcinogenic role in the breast.

References

1. Institute for Health Metrics and Evaluation, University of Washington, The Challenge Ahead: Progress and Setbacks in Breast and Cervical Cancer, September 2011. Also see: Breast and cervical cancer in 187 countries between 1980 and 2010: a systematic analysis.www.thelancet.com September 15, 2011.
2. Programme NCR. Time Trends in Cancer Incidence Rates 1982-2005. Bangalore, India: *Indian Council of Medical Research*; 2012.
3. Ali R, Barnes I, Kan SW, Beral V. Cancer incidence in British Indians and British whites in Leicester, 2004-2009. *Br J Cancer*;103:143-8.
4. Sammarco I, Capurso G, Coppola L, et al. Expression of the protooncogene C-Kit in normal and tumor tissue from colorectal carcinoma patients. *Int J Colorectal Dis* 2004;19:545-53.
5. Crisi GM, Marconi SA, Makari-Judson G, et al. Expression of c-Kit in adenoid cystic carcinoma of the breast. *Am J Clin Pathol* 2005;124:733-9.
6. Kondi-Pafiti A, Arkadopoulos N, Gennatas C, et al. Expression of c-Kit in common benign and malignant breast lesions. Tumori 2010;96:978-84.
7. Maha M. Amin, Amira K. El‐Hawary, Omar Farouk. Relation of CD117 immunoreactivity and microvascular density in invasive breast carcinoma. *Indian Journal of Pathology and Microbiology* 2012; 456-461.
8. Moritani S, Kushima R, Sugihara H, et al. Availability of CD10 immunohistochemistry as a marker of breast myoepithelial cells on paraffin sections. *Mod Pathol* 2002;15:397–405.
9. Kalof AN, Tam D, Beatty B, Cooper K. Immunostaining patterns of myoepithelial cells in breast lesions: a comparison of CD10 and smooth muscle myosin heavy chain. *J Clin Pathol* 2004;57(6):625-9.
10. Simon R, Panussis S, Maurer R, et al. KIT (CD117)-positive breast cancers are infrequent and lack KIT gene mutations. *Clinical cancer research.* 2004;10(1):178-83.
11. Bojana D jordjevic and Wedad M Hanna. Expression of c-Kit in fibroepithelial lesions of the breast is a mast cell phenomenon. Modern Pathology 2008;21, 1238–1245.
12. Tan PH, Sahin AA. Atlas of differential diagnosis in breast pathology. Springer; 2017 Apr 26.