

Distribution of Her-2/c-erb – B2 Immunostaining in Invasive Carcinomas of Breast in Mayo Hospital, Lahore

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ABSTRACT

Background: Invasive ductal carcinoma is the most frequently encountered tumour of the breast. Her-2 / Neu (c-erb-B2) oncoprotein has been demonstrated in variety of breast cancers. Its medical importance stems from its widespread over-expression in breast cancer. Over expression and magnification of Her-2 / Neu (c-erb-B2) oncogene in breast cancer patients show an early metastasis, hormonal and chemotherapy resistance along with reduced life span.

Aim: To evaluate the immunohistochemical expression of c-erb-B2 in breast tissue biopsies from patients with invasive carcinomas of breast.

Methodology: A total of 75 cases of breast cancer were included in this study. Immunohistochemical protocol was based on avidin – biotin peroxidase complex (ABC) technique of immunostaining.

Results: The results showed that out of 75 patients, 73.3% displayed no staining, 12% exhibited faint level of staining, 4% manifested moderate level and 10.7% expressed intense or marked immunostaining. The observations were tabulated and arithmetic mean was calculated.

Conclusion: IHC, or Immunohistochemistry, is a special staining process performed on fresh or frozen breast cancer tissue removed during biopsy. IHC is used to show whether or not the cancer cells have HER2 / c-erb-B2 receptors and/or hormone receptors on their surface. This information plays a critical role in treatment planning.

Keywords: Invasive ductal carcinoma, Her-2 (Herceptin), Immunostaining,

INTRODUCTION

Worldwide, the incidence of breast cancer not only is increasing but its mortality is also getting high (*Moukayed and Grant 2017*). Therefore, it is very important to find some effective therapeutic modalities. It is estimated that 1 in 9 Pakistani women will develop breast cancer at some stage of their life. In medically advanced countries there are national cancer registries where every newly diagnosed cancer patient is registered. This is quite helpful to understand the epidemiology and causative factors of cancer. In Pakistan we do not have such registry at national level. However several reports show that Pakistani women are at quite higher risk of developing breast cancer (*Farhana et al 2015*).

Histopathologically, most breast cancers are ductal cancers. Majority starts in lobules (lobular cancers) while a lesser number originate in other tissues (*Ganesh et al 2010*). Breast cancer is now not

only being considered as a single entity but a complex which has a wide variety of its molecular types, risk factors, symptoms, clinical presentation and variable responses to different treatments. Using gene expression technology, many molecular subtypes of breast cancers have been recognized. For example, estrogen, progesterone receptors, Her-2 receptors, Ki67, CA 15.3, CA 27.29 and many more (*Rick et al 2014*). There are five main molecular subtypes of breast cancer that are based on the genes a cancer expresses. **Luminal A** breast cancer is hormone-receptor positive (estrogen-receptor and/or progesterone-receptor positive), HER2 negative. Luminal A cancers are low-grade, tend to grow slowly and have the best prognosis. **Luminal B** breast cancer is hormone-receptor positive (estrogen-receptor and/or progesterone-receptor positive), and either HER2 positive or HER2 negative. Luminal B cancers generally grow slightly faster than luminal A cancers and their prognosis is slightly worse. **Triple-negative/basal-like** breast cancer is hormone-receptor negative (estrogen-receptor and progesterone-receptor negative) and HER2 negative. This type of cancer is more common in women with *BRCA1* gene mutations. **HER2-enriched** breast cancer is hormone-receptor negative (estrogen-receptor and progesterone-receptor negative) and HER2 positive. HER2-enriched cancers tend to grow faster than luminal

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cancers and can have a worse prognosis, but they are often successfully treated with targeted therapies aimed at the HER2 protein, such as Herceptin (chemical name: trastuzumab). **Normal-like** breast cancer is similar to luminal A disease: hormone-receptor positive (estrogen-receptor and/or progesterone-receptor positive), HER2 negative, which helps control how fast cancer cells grow. Hormonal receptors like estrogen, progesterone and human epidermal growth factor receptors like Her-2 has prognostic and therapeutic role and being applied in many centers of the world (*Parsa et al 2016*).

Her-2 is an epidermal growth factor receptor which is present on the cell membrane of many epithelial tissues having tyrosine kinase activity. Dimerization of the receptor results in the autophosphorylation of tyrosine residues within the cytoplasmic domain of the receptors and initiates a variety of signaling pathways leading to cell proliferation and tumorigenesis. (*Nida & Naveed 2014*). When there is mutation in protooncogenes, like translocation or gene amplification, point mutations etc, these produce specific growth signals which may lead to change in cell and ultimately the development of cancer (*Tzahar and Yarden 1998*). Her-2/ c-erb-B2 oncoprotein is linked with increased mitosis in cells, poor prognosis, recurrence of tumor and reduced survival in invasive breast cancer patients (*Daoud et al 2016*). Her-2 / c-erb-B2 is obviously a clinical success because certain antibodies can directly target amplified Her-2 / c-erb-B2 (*Charles 2012*). Even then there is no consistent evidence of a significant relationship between the immunohistochemical detection of Her-2/c-erb-B2 and epidemiological agents associated with an increased risk for developing breast carcinoma including parity, mother's age at first birth and blood relations history of breast carcinoma (*Rosen et al 1995*). A number of anti erb B2 extracellular domain monoclonal antibodies (ECD-MAb's) have been isolated and one such antibody, Herceptin, has demonstrated efficacy in the treatment of metastatic breast cancer (*Schaller et al 1999*). Therefore, the aim of this study is to identify molecular predictors like Her 2/c-erb-B2 to show whether or not the cancer cells have HER2 / c-erb-B2 receptors and/or hormone receptors on their surface. This information plays a critical role in treatment planning.

MATERIALS AND METHODS

The present study was based on immunohistochemical staining for Her-2/c-erb-B2 receptors in 75 patients of invasive breast carcinomas. The diagnosed breast tissue specimens

were randomly collected from Mayo Hospital, Lahore during the period between November 2014 till November 2016. Clinicopathological data including case number, age and biopsy type were verified from operation notes and patients registration form. The diagnosed cases of invasive carcinomas were selected irrespective of specific age. Following criteria was fulfilled to include the cases in research study.

- Confirmed tissue blocks of invasive carcinomas were included in the study.
- Mastectomy and biopsy specimens obtained by incision or excisional mean were included in the study.

Ductal carcinomas in situ (DCIS), paget's disease of the breast, borderline lesions, tissue sections showing extensive cellular distortion and cases in which diagnosis was based upon fine needle aspiration cytology (FNAC) were excluded from study. The following stains were performed on slides made from tissue blocks:

- 1: Haematoxylin and Eosin
- 2: Immunohistochemistry for Her-2/c-erb B-2

Immunohistochemical Protocol was based on the principal that an antigen is exhibited using the avidin-biotin-peroxidase complex (ABC) technique of immunohistochemistry *Bancroft and Gamble (2008)* *Beraki (2012)*. Standard microwave protocol laid down by **Biogenix** was followed. Meyer's haematoxylin is used as a counter stain. The aqueous mounting medium was used for mounting immunohistochemically stained slides and non-alcoholic stain was used for counterstaining the immunohistochemically stained slides. HER2 immunohistochemistry (IHC) score 3+ breast cancer was used as a positive control. Negative controls was obtained by excluding the primary antibody.

Assessment of Receptor Status: In the present study immunohistochemically stained slides were scored according to the criteria practiced in *North Shore Medical Centre, MA, USA (2015)*. The results were evaluated qualitatively and divided into four groups.

- "0" or no staining=negative,
- "1+" or weak staining= faint partial staining of the membrane,
- "2+" or moderate staining= weak complete staining of the membrane, > 10% of cancer cells and
- "3+" or strong= intense complete staining of the membrane, > 10% of cancer cells.
- Percentage cells positive were assessed in a semi quantitative fashion. Only membranous staining was considered as positive reactivity.

The observations were tabulated. To test the significance of difference between two variables, we compared the two totals using cross-tabulations one dimensionally, i.e., columns. All the hypotheses were tested at 0.05 level of significance

RESULTS

The results of the study are as follows:

On H & E staining, out of 75 cases, 66(88%) are diagnosed as Invasive ductal carcinoma, 1(1.3%) case was of lobular carcinoma, 2 (2.7%) cases were of medullary carcinomas, 3(4%) cases were of mucinous carcinoma and 3(4%) cases were of tubular carcinoma. Out of these 75 cases, 10(13.3%) were diagnosed as grade II carcinomas and 65(86.7%) as grade III carcinomas. Her-2 immunohistochemical staining in different types of invasive carcinomas showed no staining of any level / degree in any case of lobular, medullary, mucinous and tubular carcinomas. All cases of Her-2 immunostaining were noted in invasive ductal carcinomas (Table 1)

Though out of 66 cases of invasive ductal carcinomas, 46(61.3%) showed no (0) immunostaining, however 9(12%) cases displayed faint level (+), 3(4%) cases demonstrated moderate level (++) and 8(10.7%) cases expressed intense (+++) level of Her-2 immunostaining.

With reference to the grading of invasive carcinomas, 10(13.3%) cases were of grade II invasive carcinomas. Out of these 10 grade II tumours, 8(10.7%) cases displayed no immunostaining whereas only 2(2.7%) cases exhibited faint level of Her-2 /c-erb-B2 immunostaining. Amongst rest of the 65 (86.7%) cases of grade III invasive carcinomas, 47 (62.7%)

cases exhibited no immunostaining while 7 (9.3%) cases showed faint level, 3 (4%) moderate level and 8 (10.7%) cases manifested intense pattern of staining. Statistically, for Her-2 at level "0" and "3 +", the distribution of patients in grade III is significantly more ($p < 0.05$) than in grade II while the comparison for Her-2/c-erb-B2 at level "1+" and "2+" shows that the distribution of patients in both grades, II and III are non significant (Fig.1 & 2).

Fig. 1: Photomicrograph showing moderate (2+) level of Her-2/c-erb-B2 staining in invasive ductal carcinoma

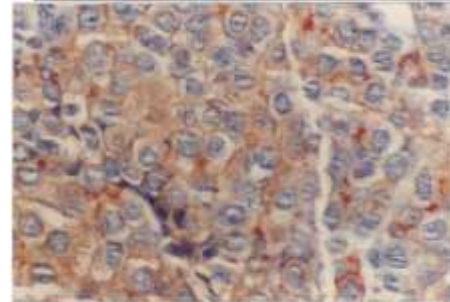


Fig. 2: Photomicrograph showing intense (3+) level of Her-2 /c-erb-B2 staining in invasive ductal carcinoma

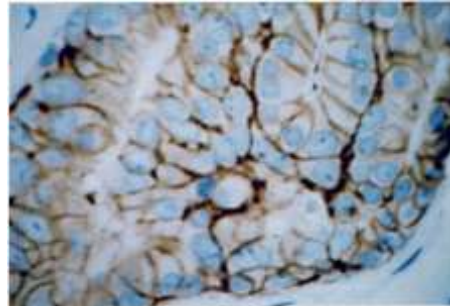


Table 1: Diagnosis on H & E

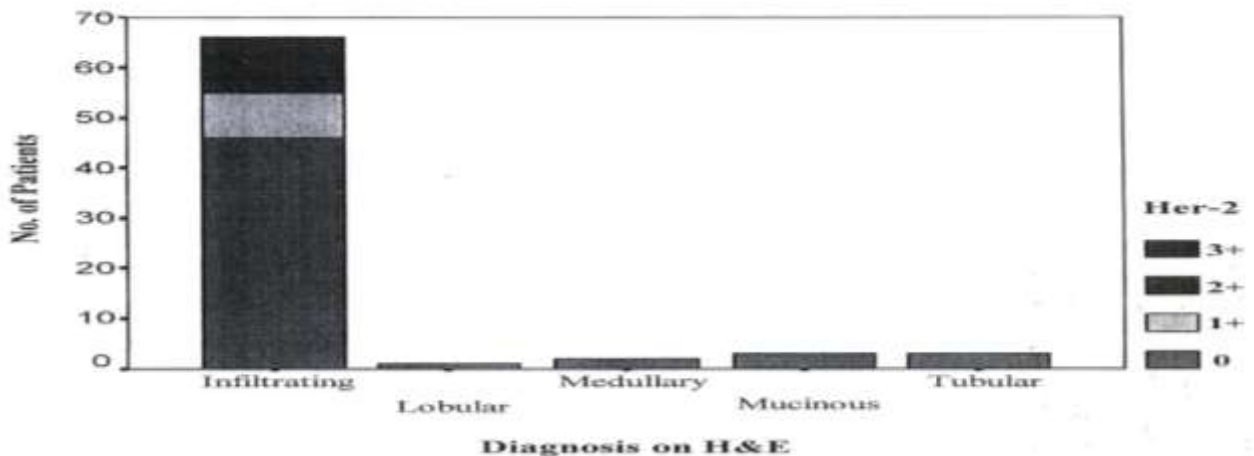


Table 2: Distribution of variable HER-2 staining patterns in invasive carcinomas (n=75)

Diagnosis on H&E	HER-2				Total
	0	1+	2+	3+	
Infiltrating ductal CA(I)	46(61.3%)	9(12%)	3(4%)	8(10.7%)	66(88%)
Lobular CA (II)	1(1.3%)	-	-	-	1(1.3%)
Medullary CA (III)	2(2.7%)	-	-	-	2(2.7%)
Mucinous CA (IV)	3(4%)	-	-	-	3(4%)
Tubular CA (V)	3(4%)	-	-	-	3(4%)
Total	55(77.3%)	9(12%)	3(4%)	8(10.7%)	75(100%)
I VS II, III, IV, V	S	S	NS	S	

HER-2=Herceptin receptor

1+=Faint staining of membrane

3+=Intensive complete staining of the membrane

0=No membrane staining observed

2+=Moderate, weak complete membrane staining

S=Significant (p<0.05)

NS=Non significant

DISCUSSION

In the present study, invasive ductal carcinoma was the commonest type of malignancy accounting 88% of total numbers. Our findings are consistent with the observations of *Ganesh et al (2010)*, *Ajithkumat & Hatcher (2011)* *Tariq et al (2015)* among whom more than 70% patients were those of invasive ductal carcinoma. This may be due to the reason that organization of breast tissue has an abundant ducts and glandular units which are undergoing active cyclical changes which are controlled by hormones of the ovarian cycle (*Guinebretière et al 2005*). In our present study, invasive lobular carcinoma, Medullary carcinoma, mucoid carcinoma and tubular carcinoma made 1.3%, 2.7%, 4% and 4% respectively. Our findings are not far away with the findings of *Chen et al 2017* and *Xin and Eng (2016)* who also found that invasive lobular carcinoma was one tenth compared with invasive ductal carcinoma as seen in 3.6% and 5 – 15% of all breast cancers respectively and makes the second commonest malignancy. The reason for this mild disparity may be a small sample size and second that invasive lobular carcinoma of the breast is more indistinct that is detected later and thus come late into the notice of patients as well as medical people. Regarding medullary and mucoid carcinomas, our findings correspond with those of *Inhye et al (2013)*, who found that medullary carcinoma is not a common variety of mammary carcinoma and *Melamed (2001)* established that mucoid carcinoma of breast is an uncommon tumour. About tubular carcinoma our findings are consistent with *Maria et al (2014)* who cited that Tubular carcinoma accounts for less than 2% of invasive breast cancers.

In the current study, all the over-expression of Her-2 immunostaining was noted in grade III tumours i.e., 4% cases showed moderate (2+) and 10.7% cases displayed intense (3+) pattern of over-expression. These results are consistent with the findings of *Looi et al (1997)* and *Hoof et al (2000)* who noted a good association between Her-2 / c-erb-

B2 over-expression and histological grade III, a known prognostic and predictive indicator of invasive breast cancer. *Grazia et al (2015)* described that tumour grade, a measure of the differentiation and mitotic activity of tumours, is a well-accepted prognostic marker in breast cancer and Her-2 / c-erb-B2 expression was associated with tumour grade. The one reason for higher number of cases with grade III invasive carcinomas may be because of late presentation by patient to medical setup. Thus Her-2 / c-erb-B2 over-expression can be expected to result in more aggressive tumour behavior and an over-expression in invasive breast tumour may be regarded as indicator of poor prognosis.

In the present study, invasive ductal carcinomas showed a frequency of Her-2 over-expression in 10.7% of cases with intense (+++) pattern and 4% cases with moderate (++) pattern. Thus overall 14.7% cases showed an over-expression with Her-2/c-erb-B2 immunostaing. *Elesawy (2014)*, *Venetia et al (2015)* and *Gallegos et al (1998)* also reported Her-2 over expression in invasive breast cancer in a range from 9 – 29.1%. Although the frequency of Her-2 expression noted in this study is towards the lower range reported in literature, this might be due to small population examined. The slight contradictions in the results of various studies can be due to various test techniques and its interpretation. Immunohistochemistry (IHC) analysis of Her 2 / c-erb- B2 is simple to perform, widely available and expensive test.

CONCLUSION

Infiltrating ductal carcinoma showed an incidence of Her-2 over-expression in 10.7% of cases with intense (3+) pattern in grade III invasive breast cancers while 4% exhibited an over-expression with moderate (2+) pattern. Thus overall, 14.7% cases showed an over-expression with Her-2 / c-erb-B2 immunostaining. The importance of Her-2 / c-erb-B2 as a prognostic, predictive and therapeutic marker in invasive breast cancer is well recognized and therefore, it is essential

to validate and standardize testing technique in order to make an accurate assessment of Her 2 status. Clinical advantages of Her 2 targeted monoclonal antibodies therapy like Trstuzumab ensures that all breast cancer patients should be tested for Her 2 / c-erb-B2 status

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