Immunohistochemical expression of Her-2/neu and its correlation with histological grades and age in IDC of breast.

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ABSTRACT:

The Her-2/neu proto-oncogene encodes a 185 kilo-dalton (Kd) transmembrane glycoprotein (p185HER2) that is a member of the epidermal growth factor receptor (EGFR) or HER family. The human epidermal growth factor Receptor 2 (HER2) might plays an important role in the prognosis of breast cancer; Hence, Her-2/neu can be the probable molecule for the study for better therapeutics of breast cancer. We analyzed paraffin embedded tissues of 165 breast cancer patients (mostly IDC NOS). These cases were divided into three grades according to scarf bloom Richardson grading system. The expression of Her-2/neu was studied by immunohistochemistry by using purified mouse anti-human Monoclonal antibody. In our study we find that the age ranges between 18 years and 73 years. The mean and median age of breast cancer was 48.69 years and 48 years respectively with peak prevalence rate in the age group of 45 years to 60 years. Secondly, we found that 46.6% of cases were positive for Her- 2/neu and in histological grade wise it was positive for Her 2/neu in 14/35, 41/91 and 22/39 cases in grade I, grade II, and grade III tumors respectively. In other words, 40%, 45.05% and 56.41% cases were positive for Her -2/neu expression amongst grade I, grade II and grade III tumors respectively. Thirdly, we also found that Her 2/neu expression is increases with the increase age of the

patient. Our study shows Her-2/neu expression was positively related to histological grade as well as with the age of the patient. Although the difference in expression rate across different grades was not statistically significant.

Key words: Immunohistochemistry, Her-2/neu, breast cancer.

Introduction:

The HER2 proto-oncogene encodes a 185 kilo-dalton (Kd) transmembrane glycoprotein (p185HER2) that is a member of the epidermal growth factor receptor (EGFR) or HER family (Coussens et al. 1985, Yamamoto etal.1986). The HER2 gene is located on the long arm of chromosome 17 (17q11-q12) (Schechter et al. 1985) and spans the cell membrane with a large extracellular domain (ECD; 632 aa), a short hydrophobic transmembrane domain (TM; 22 aa) and an intracellular cytoplasmic domain (ICD; 580 aa) containing both a tyrosine kinase domain, and carboxy terminal domain that is autophosphorylated upon receptor activation (Choudhury and Kiessling 2004). This receptor is expressed on the cell membrane of a variety of epithelial cell types and through binding of specific growth factors, regulates aspects of cell growth and division. The designation "neu" originated from the rat neu gene that was identified first in N-ethyl-N-nitrourea chemically induced neuroblastomas and glioblastomas in rats (Padhy et al. 1982, Drebin et al. 1984, Schechter et al. 1984). HER2 is the human homologue of the rat neu protein, with human and rat homology being around 89% when comparing the nucleic acid and aa sequences. From now on the designation HER2 will be used when referring to the gene and its gene product in general terms. In human tissues amplification of HER2 is found in association with various adenocarcinomas especially mammary carcinomas and found that amplification of

HER2 was at least three times more frequent in breast cancer than in other types of carcinomas (Yokota et al 1986). In breast cancer the HER2 gene is amplified in approximately 20% to 25% of all cases such that instead of having two copies of the gene per cell (one on each chromosome 17), there may be as many as 50 or 100 HER2 gene copies per cell (Slamon DJ et al 1987). This gene amplification even results in over expression of p185 Her2/neu at both the transcript and protein levels; there can be as many as approximately 2,000,000 Her2/neu molecules per cell in malignant tissues, instead of the normal compliment of approximately 20,000 to 50,000 molecules per cell (Slamon, 2000). When Her2/neu is over expressed at these abnormally high levels, the kinase activity becomes constitutively activated possibly auto-activation caused by crowding of adjacent Her2/neu receptor molecules within the cell membrane (Sharpe S et al 2002). The HER2 gene, present as a single copy in normal epithelial cells, is amplified by gene amplification in numerous malignant cell types, and its overexpression may contribute to disease initiation and progression (Yarden 2001). In humans, HER2 is frequently amplified or overexpressed in many types of cancers, including ovary (Slamon et al. 1989, Meden and Kuhn 1997), lung (Weiner et al. 1990,), stomach (Yokota et al. 1988, Kono et al. 2002a), oral (Xia et al. 1997, Xia et al. 1999), and breast (Slamon et al. 1987, Slamon et al.1989, Gusterson et al. 1992, Toikkanen et al. 1992, Ross and Fletcher 1999) cancers. Overexpression in breast cancer has, in general, been associated with more aggressive disease and a poor prognosis (Semba et al. 1985, Slamon et al. 1987). This alteration is the result of a somatic (non-inherited) event occurring sometime during the life of the patient for reasons that are still unclear. When the HER2 gene is overexpressed at these abnormally high levels, its kinase activity is similarly increased, which in turn initiates signal transduction resulting in either cellular proliferation and/or differentiation, depending on the ligand as well as the conditions (Wen et al. 1992, Falls

et al. 1993, Reese and Slamon 1997). This is possibly due to auto activation caused by crowding of adjacent HER-2/neu receptor molecules within the cell membrane (Reese and Slamon 1997). Human breast and ovarian cancer have been shown to have a number of substrates for the HER-2/neu tyrosine kinase containing SH2 and SH3 domains. Consequently, the immediate early nuclear transcription genes including c-fos, c-jun,and c-myc are rapidly upregulated (Graus-Porta et al. 1995, Carraway et al. 1999, Menard et al. 2000). Ligand-induced HER1 signaling is normally regulated by receptor down regulation that is controlled by endocytosis, endosomal sorting, and lysosomal targeting (Sorkin and Water 1993, French et al. 1994). HER1 is rapidly endocytosed and degraded in response to activation by EGF. This is mostly controlled by targeting to lysosomes (Worthylake and Wiley 1997, Lenferink et al. 1998).The restricted overexpression of HER2 to tumors, particularly those of epithelial origin, and the direct contribution of Her-2/neu to the ontogeny of certain malignancies like breast cancer make this molecule an attractive target for anticancer therapy. The aim of the present study was to assess the expression pattern of Her 2/neu in various histological grades and its correlation with age in the IDC of breast.

Material Methods

The present retrospective study was conducted in the Centre for Physiotherapy & Rehabilitation Sciences, Jamia Millia Islamia, New Delhi and Department of Pathology, Maulana Azad Medical College, New Delhi. All the diagnosed cases of carcinoma of breast which came to the Department of Surgery at Lok Nayak Jai Prakash Hospital, New Delhi for treatment were selected. These cases underwent either conservative breast surgery or radical mastectomy and the excised specimen was received in the Department of Pathology, Maulana Azad Medical College,

New Delhi. Here all the specimens were processed and paraffin blocks were prepared for histological examination. The required tissue sample for the presents study was retrieved from those paraffin blocks, which were prepared from Primary breast tumor site only those cases, which were diagnosed as infiltrating ductal carcinoma, not otherwise specified (IDC, NOS) were included in the study.

HISTOPATHOLOGY:

All the cases were routinely processed and Hematoxylin & Eosin staining was done for making histological diagnosis. Only cases of IDC, NOS were included in the present study. All these cases were graded into one of three histological grades using Scarf-Bloom-Richardson grading system.

IMMUNOHISTOCHEMISTRY:

Immunohistochemistry is the localization of antigens in tissue sections by the use of labeled antibodies as specific reagents through antigen-antibody interactions. These were as follows: Her 2/neu, Purified mouse anti-human

Monoclonal antibody (Novacastra,USA)

Secondary antibody (Novacastra,USA)

Tertiary antibody (Novacastra,USA)

DAB (Novacastra,USA)

The Immunohistochemical stains were performed using Avidin – Biotin technique.

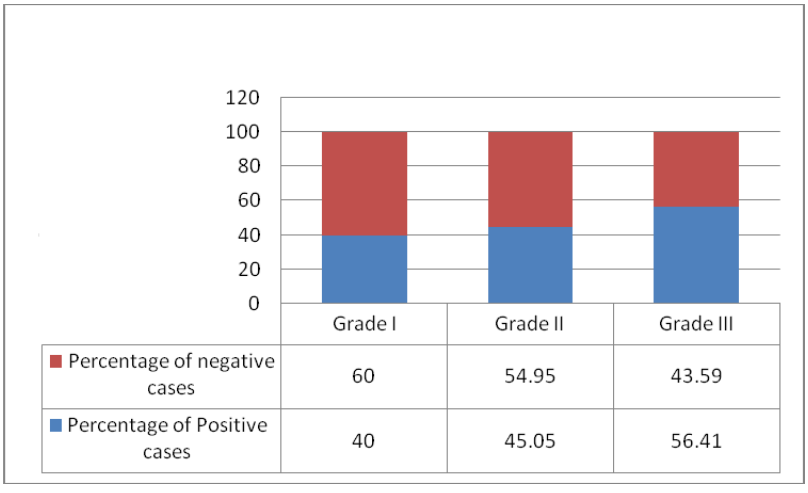
Interpretation of Immunohistochemical stains:

Immunohistochemically stained histolgical sections were studied under light microscopy. Slide was screened for localizing tumor area. Whole tumor area was observed and overall percentage positivity of tumor cells for Her-2/neu was counted under x400 magnification.

Result:

(a) Immunohistochemical expression of Her-2/neu onco-protein in histological grades.

Out of 165 cases of breast cancer, 77 (46.66%) cases showed positive staining for Her-2/neu onco-protein. These cases were further divided into three histological grades according to histological features. It was found that Her- 2/neu expression was positive in 14/35, 41/91 and 22/39 cases in grade I, grade II, and grade III tumors respectively (Figure1 & 2). In other words, 40%, 45.05% and 56.41% cases were positive for Her- 2/neu expression amongst grade I, grade II and grade III tumors respectively. Thus, maximum positivity was seen in grade III while least positivity was seen in grade I tumors (Figure1 & 2). The immunohistochemical expression of Her-2/neu showed positive correlation with histological grade, although, the distribution pattern is statistically not significant ($p<0.19$).



	Grade I	Grade II	Grade III
Percentage of negative cases	60	54.95	43.59
Percentage of Positive cases	40	45.05	56.41

Fig. 1 Percentage of Immunohistochemical expression of Her 2/neu onco-protein in different histological grades (n=165).

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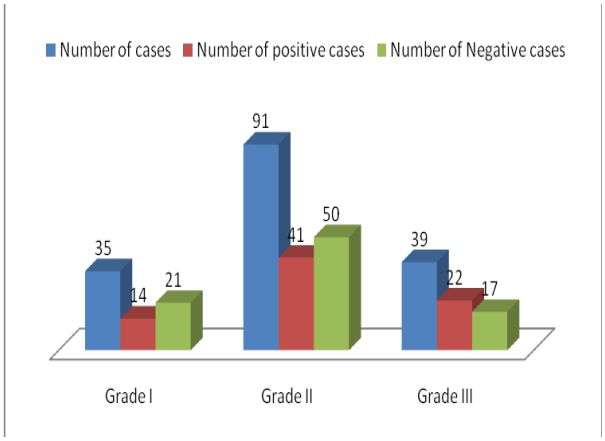
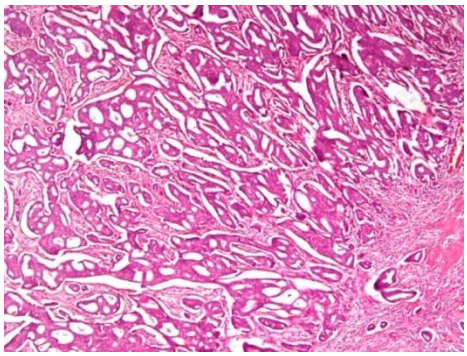
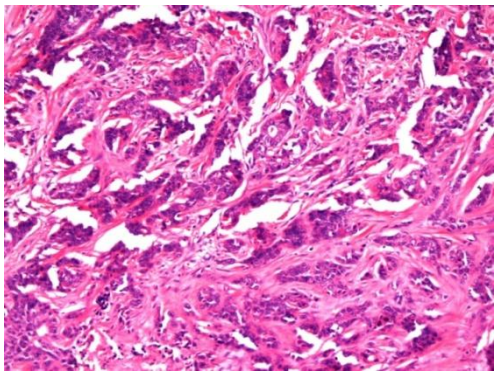


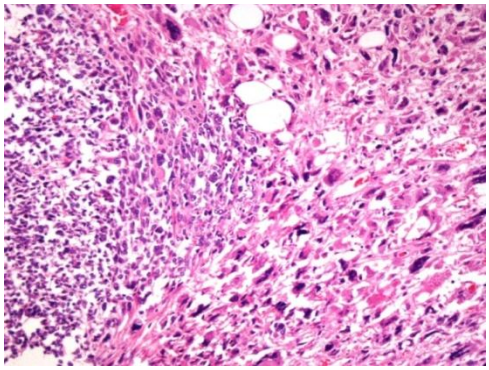
Fig. 2: Distribution of cases (number) for immunohistochemical expression of pro-apoptotic Bax protein in different histological grades (n=165)



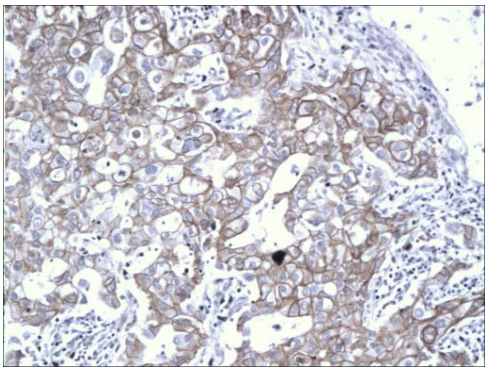
H&E, x200. Tumor shows tubule formation; no solid area or necrosis is identified (IDC, NOS; grade I)



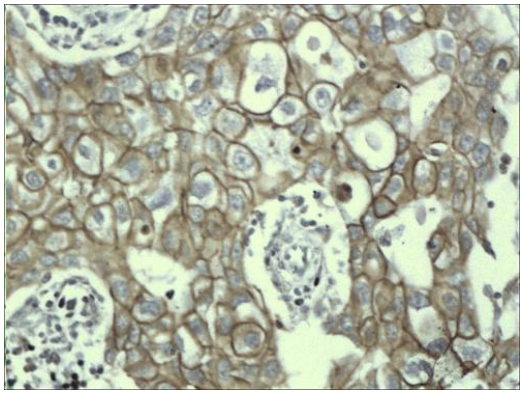
H&E, x400. Tumor shows tubule formation as well as solid area; no tumor necrosis is identified (IDC, NOS; grade II)



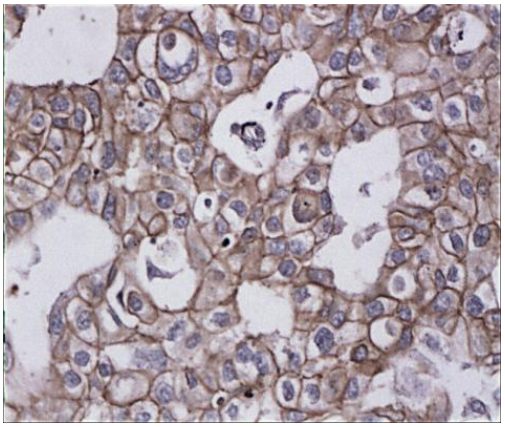
H&E, x400. Tumor shows solid architecture with high grade nuclear feature showing nuclear pleomorphism and occasional mitotic figures (IDC, NOS; grade III)



Her-2/neu IHC, x200. Her-2/neu positivity in grade I tumors



Her-2/neu IHC, x400. Her-2/neu positivity in grade II tumors



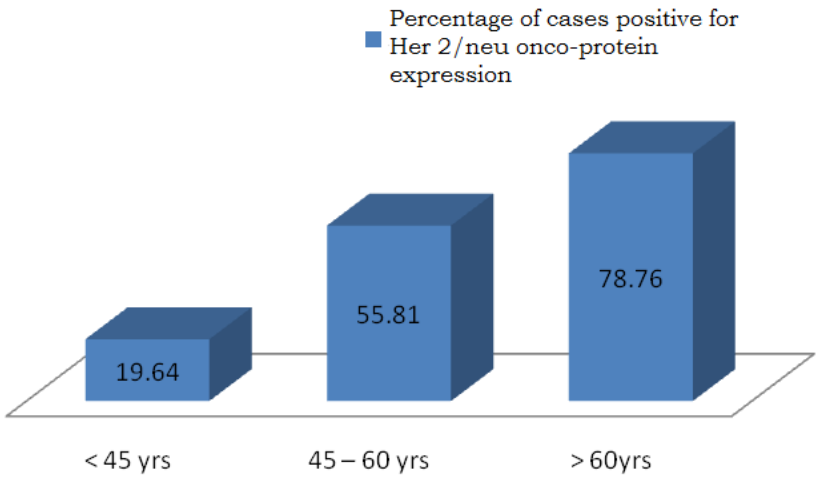
Her-2/neu IHC, x400. Her-2/neu positivity in grade III tumors

(b) Relation between expression of Her-2/neu onco-protein protein with Age:

A total of 165 cases of breast carcinoma were divided into three age groups i.e. cases having age < 45 years, cases having age between 45 years to 60 years and cases having age > 60 year. It was found that maximum 78.76% (18/23) of cases were positive for Her-2/neu expression in the age group > 60 years, while least 19.64% (11/56) of cases was positive for Her-2/neu expression in the age group < 45 years. The age group of 45 years to 60 years showed 33.72 % (48/86) of cases were positive for Her-2/neu expression **(Table 2).** The pattern of Her-2/neu expression is positively progressively correlated with age i.e. with the increasing of the age her 2/neu expression is increasing. However, this association is not statistically significant (p=0.14).

Age Group	No. of positive cases for Her-2/neu	Total number of cases in the Age group	Percentage of positivity for Her-2/neu
< 45 yrs	11	56	19.64
45 – 60 yrs	48	86	55.81
> 60 yrs	18	23	78.76

Table 2: Relation of Her-2/neu onco-protein expression with different age groups (n = 165)



Distribution of cases (percentage) for immunohistochemical expression of Her 2/neu onco-protein expression in different age groups (n = 165)

Discussion:

Breast cancer remains the most common malignancy in women worldwide and is the leading cause of cancer-related mortality. In the recent past, it has shown an increase trend of prevalent rate, exceptionally in urban location of developing and less developed countries (Hortobagyi GN de la Garza Salazar J et al, 2005). Hence, because of its much prevalence in developing countries, we decided to work on breast cancer prevention and probable molecules that can be used for future therapeutics. The aim of this study was to assess the expression of the Her-2/neu oncoprotein in various histological grades and its correlation with the age in IDC of breast. Breast cancer is a heterogeneous disease with variable biological and clinical characteristics. Age and female sex are one of the major risk factors for breast cancer, with incidence rates rising rapidly between the ages of 35 and 39 years and subsequently leveling to a plateau after 80 years. (Anderson WF et al, 2005) In the present study, all 165 cases of breast carcinoma were distributed in the range of 18 to 73 years and the peak prevalence rate of cases was seen in the age group of 45 to 60 years of age (52.12% of total cases) which is consistent worldwide. Least number of cases was localized in age group of more than 60 years of age. Hence the prevalence rate showed a decreasing trend with increase in the age of patients. Nonetheless, the rate of increase slows around the age of 50 years, corresponding to the average age of menopause. (Clemmesen J et al, 1948). The mean age in our study comes out to be 48.69 years and the median is 48 years. According to National cancer registry programme, hospital based cancer registry, 2006 the mean age is less than 50 years in Indian population while according to National cancer Institute, USA, the median age at diagnosis for breast cancer is 61 years (Altekruse et al, 2009).In the present study, On light microscopic examination, all the cases were grouped into one of three histological grades. As per Scarff-Bloom-Richardson histological

grading system, maximum number of cases was categorized in grade II (55.15%) while grade I and grade III cases constituted 21.21% and 23.64% respectively. This type of gradewise distribution of cases has been seen in other studies also, present study has included only IDC, NOS type of breast cancer which itself shows predominantly grade II cases of breast carcinoma. (Elston, C.W. et al, 1991; Dalton, L.W et al, 2000). Histological grading is an established independent prognostic factor. In a series of 1262 cases Le Doussal V et al, 1989 have found prognostic significance of histological grade of breast carcinoma. Her-2/neu, an oncogene, which has been found to be most commonly associated with breast cancer pathogenesis as well as prognosis (Slamon DJ et al, 1987). In the present study, overall expression rate of Her-2 /neu was 46.66%. This was slightly higher than as seen in most of other studies where they have reported its expression from 10 to 40% (Slamon, DJ et al,1987; Mansour EG et al,1994; Schmitt FC et al, 1995). According to histological grade, its expression varied from 40% (14/35) in grade I tumors to 56.41 %(22/39) in grade III tumors. Thus, Her 2/neu expression was positively related to histological grade, although the difference in expression rate across different grades was not statistically significant (p=0.19). Slamon DJ et al, 1987; Slamon DJ et al, 1989; Rehman S et al, 2000; Ross JS et al, 2003; also showed very similar positive correlation between Her-2/neu expression and histological grades in their respective studies. This positive correlation between Her-2/ neu expression and histological grade accounts for the poor prognostic significance of Her-2/neu in case of breast carcinoma. The expression of Her 2/neu also varies with age of the patient. Maximum positivity for Her-2/*neu* was found in the age group of more than 60 year and least in age group of < 45 year age of the patient. However, this correlation was statically not significant. Earlier no evidence is reported regarding the Her2/neu expression and age of the patient. Based on these findings of our study it may be suggested that Histological

grading is an important independent prognostic marker while Her-2/neu expression levels and its association with the age may also have significant role in the prognosis. It might require large number of studies for establishing its significant role and it can be a target for successful management of breast cancer.

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