

Original article

Correlation of HER- 2 over expression and tumour grade in ductal carcinoma breast: A three years study in a tertiary teaching hospital in Uttar Pradesh, India

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Abstract:

Breast cancer is a heterogeneous disease composed of multiple subtypes. Infiltrating ductal carcinoma (not otherwise specified-NOS) is the most common type. This study was designed to detect HER -2 expression by immunohistochemistry on routine sections of breast carcinoma cases and to compare it with the histological grading of ductal carcinoma.

Keywords:

Immunohistochemistry, Breast cancer, Molecular biomarkers, Histopathology, Oncology

Introduction

Breast cancer is the second most common cancer among Indian women and there is an increase in trend in metropolitan cities. The age adjusted incidence rate (AAR) of breast cancer in India varied from 6.8 to 33 per 100000 women as per the annual report of the National cancer registry program under the ICMR.^[1] Many factors have been implicated in the prognosis of breast cancer. Age of patients, menopausal status, family history, tumour size, grade, lymph node status and distant metastasis are among the numerous factors implicated. At the molecular level, three molecular biomarkers are routinely used in the clinical management of patients with invasive breast cancer: Estrogen receptor(ER), Progesterone receptor (PR), and human epidermal growth factor receptor-2 (HER-2). Since all are targets and indicators of highly effective therapies against breast cancer, accurate assessment is mandatory.^{[2,}

^{3]}

HER 2/neu (ErbB-2) is an oncogene that encodes a trans membrane glycoprotein with tyrosine kinase activity.^[4] The proto-oncogene HER-2/neu has been localized to chromosome 17q(17q21-q22). HER 2 was so named because it has a similar structure to human epidermal growth factor receptor, or HER1. “Neu” was so named because it was derived from a rodent glioblastoma cell line, a type of neural tumour. Name “ERbB-2(v-erb-b2 erythroblastic leukemia viral oncogene homolog 2, neuro/glioblastoma derived oncogene homolog - avian)” was derived for its similarity to ERbB (avian erythroblastosis oncogene B) gene.^[5]The HER-2/neu protein is a component of a four member family of closely related growth factor receptors, including EGFR or HER-1 (erb-B1); HER-2 (erb-B2); HER-3 (erb-B3) and HER-4(erb-B4). Signalling through the ErbB family of receptors promotes cell proliferation and opposes apoptosis.^[6]

HER 2 expression has been evaluated in invasive breast cancers for about 25 years. Amplification or over-expression of the HER 2 gene occurs in approximately 15 % of primary breast cancers. Amplification is positively correlated with increased protein expression.^[7] The relationship between HER 2 status and clinical outcome is complex and varies with settings. Recent studies demonstrate that HER 2-positive invasive breast carcinoma cases respond favourably to therapies that specifically target the HER 2 protein (eg. trastuzumab and lapatinib).^[8] The main reason for assessing HER 2 status is to identify candidates for targeted therapy.

HER 2 positivity in tumours are graded as per the grading system devised by the American society of clinical oncology. It is strongly associated with increased disease recurrence and a worse prognosis^[9] Over-expression is also known to occur in other cancers (ovarian, stomach, and uterine serous endometrial carcinoma).^[10] The role of HER 2 in tumour genesis may be due to formation of clusters of HER 2 proteins in cell membranes.^[11] In recent years, with the advancement in molecular and genetic studies, this protein has become an important biomarker. It is now a well established target of therapy for approximately 30% of breast cancer patients.^[12]

The monoclonal antibody trastuzumab (marketed as Herceptin) is targeted against HER 2. Trastuzumab is effective solely in cancers with HER 2 over-expression. An important downstream effect of trastuzumab is stopping the cellular proliferation. This occurs by an increase in p27.^[13] Breast tumours are routinely checked for over expression of HER 2/neu because of its prognostic role as well as its ability to predict response to trastuzumab.

Material and methods

The Present study was designed with the objective of assessing the histological grading of infiltrating ductal carcinoma breast and correlating it with the over expression of Her 2 immuno-reactivity. This study was conducted in the department of Pathology of MLB government medical college Jhansi, Uttar Pradesh for three years- from May 2010- April 2013. Sample size: A total of 54 cases of confirmed breast cancer (both prospective and retrospective) were included in the study in which adequate tissue material and clinical data was available.

Inclusion criteria: All the confirmed cases of carcinoma breast during the specified period of four years were included in the study.

Exclusion criteria: 1) Non-availability of representative tissue of the tumour

2) Poor tissue processing.

3) Cases not falling in between the specified period of time duration.

This study was both retrospective and prospective. For retrospective study blocks and slides from the histopathology section of the department were obtained. For prospective study fresh breast mastectomy specimens, core biopsy material obtained from surgery department submitted for histopathological examination were included in the study.

- Biopsies and mastectomy specimens were fixed in 10% formalin.
- Detailed history about age, residence, clinical diagnosis and chief complaints was enquired in prospective cases.
- Gross appearance of mastectomy specimen/biopsy was noted. Paraffin blocks after proper tissue processing were

prepared. 3-4 micron thick specimen sections were cut.

- Routine haematoxylin and Eosin staining was done for histo-morphological typing and histological grading of all cases of infiltrating ductal carcinoma. Histological grading of infiltrating ductal carcinoma was done according to grading system devised by Elaston and Ellis (1991), which includes tubule and gland formation, nuclear pleomorphism, and mitotic counts.^[14]
- Immunohistochemistry was done using labelled antibodies for HER 2 gene. Both negative controls (sections incubated without primary antibody in TRIS buffer) and positive controls (known positive cases) were applied. After staining the slides, results were viewed under ordinary light microscope, grading was done on the basis of grading system devised by the American society of clinical oncology. **(Table 1)**

Results

A total 54 cases of breast cancer were studied. Maximum numbers of the patients belong to 41 to

50 years of age group, followed by 51-60 years of age group. Mean age at surgical intervention being 48 years. Out of total 54 cases studied, maximum 48 (88.9 %) cases were of invasive ductal carcinoma (NOS), followed by 02 (3.70%) cases of mucinous carcinoma, 02 case of medullary (3.70%), 01 cases of sarcoma phylloides (1.85%) and 01 case of metaplastic carcinoma (1.85%). The 48 cases of invasive ductal carcinoma (not otherwise specified-NOS) were submitted for the histological grading. Scores obtained were used for grading the tumour. **(Table 2)** Only 12 (25.0%) were of grade I with score 3-5. Maximum 21 (43.75%) cases belonged to grade II with score 6-7 followed by 15 (31.25%) cases were of grade III with score 8-9. **(Image 1 and 2)** Sections of all the cases (n=54) were cut for HER-2 assessment. Results obtained were correlated with tumour grades in case of ductal carcinomas. Out of total 54 cases of breast carcinoma, only 09 cases show HER 2 immunostaining. (16.67%) **(Image 3 and 4)** Out of 09 cases, only one case(8.33%) belong to grade I(1 out of 12 cases of grade I), 04 cases(19.05%) belong to grade II(04 out of 21 cases of grade II) and 04 cases(26.67%) belong to grade III(04 out of 15 cases of grade III).

Table 1
Grading of the immunohistochemical staining for HER 2 over expression

The American Society of Clinical Oncology/ College of American Pathologists (ASCO/CAP) guidelines.

Staining	Score	HER 2/neu protein over expression assessment
No staining is observed or membrane staining is observed in less than 10% of the tumour cells	0	Negative
A faint/barely perceptible membrane staining is detected in more than 10% of the tumour cells. The cells are only stained in part of their membrane.	1+	Negative
A weak to moderate complete membrane staining is observed in more than 10% of the tumour cells	2+	Weakly Positive
A strong complete membrane staining is observed in more than 30% of the the tumour cells.	3+	Strongly positive

Table 2

Histological case wise distribution of cases of ductal carcinoma

Grade	Number of cases	Percentage
I	12	25.00%
II	21	43.75%
III	15	31.25%

Table 3

Correlation of Her 2 immuno-reactivity with histological grades

Grade	Number of cases with Her 2 positivity	Percentage
I (n =12)	01	8.33%
II(n=21)	04	19.05%
III(n=15)	04	26.67%

Table 4

Comparative results from various studies

Studies	HER2 positivity in grade I Carcinomas	HER2 positivity in grade II carcinomas	HER2 positivity in grade III carcinomas
Present study	8.33%	19.05%	26.67%
Lal et al [21]	0%	10%	28%
Rilke et al [23]	3.9%	20.4%	38.9%
Tsuda et al [24]	0%	10%	33%
Hoff et al [25]	1%	17%	23%

Image 1

Photomicrograph of histopathology section of Grade II ductal carcinoma showing tubule formation (H&Ex400)

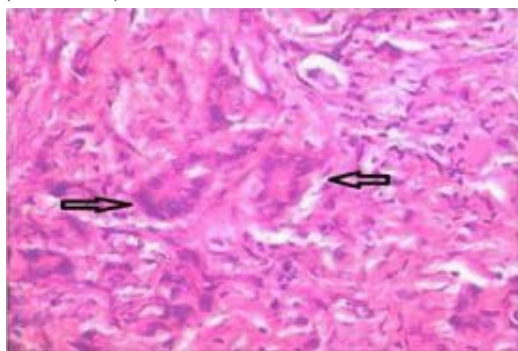


Image 2

Photomicrograph of histopathology section of Grade III ductal carcinoma showing atypia and bizarre nuclear features.(H & E x400)

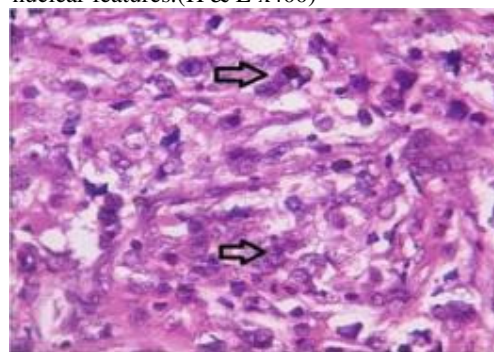


Image 3

Photomicrograph of HER- 2 immuno-reactivity for membranous staining score 3+ (x100)

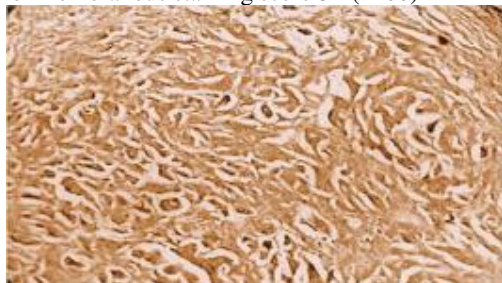
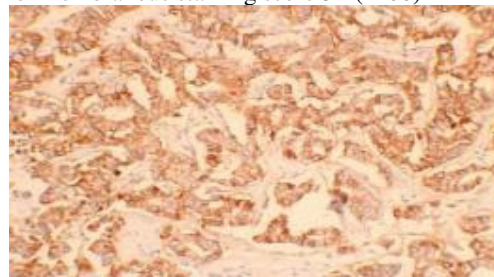


Image 4

Photomicrograph of HER- 2 immuno-reactivity for membranous staining score 3+ (x400)



Discussion

HER-2 is one of the important predictive factors associated with breast cancer. HER-2 gene is amplified and over expressed in approximately 15-40% of breast cancers. It correlates with an unfavourable outcome. It is associated with a shorter disease free interval, relative resistance to hormonal agents, and tendency to metastasize to the brain and viscera. With the advent of newer anti-cancer drug Trastuzumab, prognosis has greatly improved in such patients as the amplified breast cancers have increased sensitivity to certain cytotoxic agents such as doxorubicin.^[15] These observations were derived from the studies designed to predict the response of various therapies for cancer treatment (hormonal therapies, cancer chemotherapy, and radiotherapy) in context to HER-2/neu status.^[16]

In the present study 54 cases of malignant lesions of the breast were included. Most of the patients belong to 40 to 60 years of age group. Mean age at presentation and diagnosis being 48 years. In a study on 165 cases, the mean age at diagnosis was 52.5 ± 12.1 years.^[17] Out of these 54 cases, 48 (88.9 %) were invasive carcinomas of epithelial origin. One case (1.85%) was of stromal tumour (sarcoma phylloides). These findings were in accordance with the study of Hussain et al. who noticed in his study that there was 97.6% of epithelial and 2.4% of stromal tumours.^[18] Berg and Hutter^[19] also stated that the commonest were the neoplasms arising from the epithelial component, which constitute the glandular element of the breast. According to them the most common type of carcinoma is infiltrative ductal carcinoma (70%). In the present study, all invasive ductal carcinomas were graded according to the Modified Bloom Richardson Grading by Elston and Ellis. Out of the 48 cases of ductal carcinomas, 43.75% were Grade II, 31.25% were Grade III while 25.0% were

Grade I (**Table 2**). According to a study by Seshie B *et al.* tumour grades were as following- Grade I 8.3 %, Grade II 60.8 % and Grade III 30.8%. Our results closely matched with the study of Doussal et al.^[20] They studied on 1262 patients, 11 to 14% were grade I, 55 to 57% were grade II and 29-34% were grade III. Out of 54 cases HER- 2 positive immune-staining was seen in 09 cases. This gives HER2 expression in 16.67% cases. Lal P. et al found HER2 over expression in 15%-25% of invasive breast carcinoma.^[21] Our results may be lower as we encountered a fair number of Grade I cases.

Of the 09 immuno-reactive cases, only one case (8.33%) belong to Grade I (1 out of 12 cases of Grade I), 04 cases (19.05%) belong to Grade II (04 out of 21 cases of Grade II) and 04 cases (26.67%) belong to Grade III (04 out of 15 cases of Grade III). (**Table 3**) There are a many studies by different researchers on HER2 over expression compared with tumour grading of infiltrating ductal carcinoma of breast. The results were comparable in most studies. (**Table 4**) Goud *et al.*,^[22] studied 90 cases, out of which 28 cases were with the score of 2+ (grade II), 32 cases with score 3+ (more case of 3+ grade followed by 2+ grade) rest of the cases were score 1+ or 0 (no staining).

Our results closely matched with the study by Rilke *et al.*^[23] The correlation between HER-2 expression and tumour grade was studied in a large series of 1,210 cases by Rilke *et al.*, who found HER-2 over expression rates of 3.9%, 20.4%, and 38.9% in tumours of grades 1, 2, and 3, respectively. Tsuda *et al.*^[24] found HER-2 amplification in 0% of grade 1 invasive ductal carcinomas, 10% of grade 2 ductal carcinomas, and 33% of grade 3 ductal carcinomas. More recently, Hoff *et al.*^[25] in a study of 388 cases found that HER-2 was amplified in less than 1% of grade 1, 17% of grade 2, and 23% of grade 3 tumours.

Similarly, in one study Lal *et al* showed HER-2 positivity in 0% of grade 1 invasive ductal carcinomas, including tubular carcinomas, approximately 10% of grade 2 ductal carcinomas, and almost 28% of grade 3 ductal carcinomas. HER-2 was positive in 10.87% of grade 2 and 27.84% of grade 3 ductal carcinomas and negative in all grade 1 ductal carcinomas. HER-2 over expression or amplification essentially was limited to grades 2 and 3 ductal carcinomas and correlated inversely with ER or PR expression. There were two main limitations of this study which we experienced. One was due to the limited number of samples. If the sample group could be increased the results would have been better. Secondly, the antigen retrieval from the paraffin blocks of older cases was a difficult task. The antigen retrieval was much better in cases of newer blocks. Many studies have been conducted on this topic earlier. HER-2 is now a well established molecular bio-marker in

evaluating carcinoma breast in big cities but the small cities and towns are lacking in diagnostic set-ups and procedures. This result in referral of patients to higher centers in big cities and increase work-load of these referral centers. The main clinical implication of this study is to lay emphasis on the introduction of HER-2 as a routine molecular bio-marker in study of carcinoma breast even in small cities of Uttar Pradesh. This will definitely improve the patient survival and increase disease free interval.

Conclusions

Invasive ductal carcinoma (NOS) was the commonest type of histological pattern. HER2 expression was seen in 16.67% cases. HER-2 over expression was more encountered in grade III tumours. HER-2 over expression or amplification essentially was seen mainly in grades 2 and 3 ductal carcinomas. Grade I carcinomas had low positivity.

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