

HER-2/neu overexpression in breast cancer

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Summary:

Background: In breast carcinoma, amplification &/or over-expression of HER-2/neu has been associated with a group of unfavorable prognostic factors. The Food & Drug Administration Agency approved Trastuzumab (Herceptin) for the therapy of metastatic breast cancer but only in patients with amplification &/or over-expression of this gene. Because of these advances, evaluation of HER-2/neu status in breast cancer specimens is of vital importance.

Patients and methods: thirty eight females with breast carcinoma were included in this study. All histologic sections were stained routinely with the hematoxylin and eosin stains and immunohistochemically for HER2/neu. All cancers were graded and subcategorized into ductal and lobular carcinomas, with or without an insitu component, and with or without Paget disease. The assessment of HER-2/neu over-expression was quantitated.

Results: Of the twenty four cases of infiltrative duct carcinoma (IDC), HER-2/neu staining was positive in ten (41.7%) cases. None of the fourteen cases of infiltrative lobular carcinoma (ILC) showed a positive reaction. Statistical analysis revealed no significant differences in respect to HER2/neu expression between IDC with and without ductal carcinoma in situ (DCI). All cases of IDC with overlying Paget disease (5 cases; 13.2%) were positive for the stain. All grade I cancers were negative for HER-2/neu over-expression, in contradistinction to a positive staining in 41.7% grade II tumors and 62.5% grade III tumors.

Conclusion: there is significant correlation between HER2/neu overexpression and cancer histopathological type. The presence or absence of an in situ component had no effect on the marker overexpression. Significant correlation was observed between HER-2/neu overexpression and higher histological grades of IDC. No correlation was found between the positivity of the marker and axillary lymph node status. Similarly, no association was found with the other variables except the size of tumor. It is recommended that HER-2/neu assessment to be included as a routine test in the screening program of all breast cancer Iraqi patients.

Key words: HER-2/neu, breast carcinoma, prognostic variables

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Introduction:

One of the most common genetic alterations associated with human cancers, including that of the breast, is the amplification of HER-2/neu proto-oncogene which is located on chromosome 17q11.1. 2 HER-2/neu (also called c-erbB₂) oncogene encodes for a trans-membrane glycoprotein with tyrosine kinase-activity that belongs to the family of epidermal growth factor receptors 3. In the context of breast carcinoma, an association has been found to exist between the amplification &/or over-expression of HER-2/neu and a group of unfavorable prognostic factors that include large tumor size, higher histologic grade, lack of steroid receptors expression, presence of axillary lymph nodes metastasis, early relapse, as well as reduced overall survival.⁴ All the aforementioned variables are in fact direct or indirect determinants of advanced stage. The Food & Drug Administration Agency approved Trastuzumab (Herceptin) for the therapy of metastatic breast cancer. Only patients with gene amplification and/or over-expression are eligible for this treatment⁵. Because of these advances, there is a compelling need to evaluate HER-2/neu status in

breast cancer specimens of Iraqi patients who might benefit from such a therapy.

Patients, materials, and methods:

From January 2008 through March 2009, thirty eight females with breast carcinoma; attending the Al-Yarmouk Teaching Hospital, were included in this study. These were represented by samples of radical mastectomy (17 patients) and excisional biopsy (21 patients). All tissue samples retrieved were formalin fixed-paraffin embedded blocks. Five micron-thick sections were obtained from the tissue blocks, which were subsequently stained with the routine hematoxylin and eosin stains⁶, as well as immunohistochemically for HER2/neu through streptavidin biotin staining methods⁷. Hematoxylin and eosin sections of the submitted carcinomas were examined for the confirmation of the histological type of carcinoma and to determine the grading (according to Nottingham modified Bloom-Richardson Grading Scheme)⁸ Positive (Prostatic tissue) and negative controls (carcinomatous breast samples after removal of the primary antibody) were run with HER2/neu staining of the cases studied. Accordingly, the carcinomas in this study were sorted out according to the following categories

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- Invasive ductal carcinoma without in situ component (IDC)
- Invasive ductal carcinoma with an in situ component (IDC + DCI)
- Invasive ductal carcinoma with overlying Paget disease (IDC + PD)
- Invasive lobular carcinoma without an in situ component (ILC)
- Invasive lobular carcinoma with in an situ component (ILC + LCI)

The relevant clinicopathological data were obtained by reviewing the medical files and pathological reports of the patients, which included information regarding age at presentation, size of the cancer and axillary lymph node status. The assessment of HER-2/neu over-expression was quantitated according to the number of stained cells, as well as the pattern and intensity of staining; this was done through a score of 0 to 3+ as shown in table 1

Table 1: Scoring of HER2/neu staining*

No. of +vely stained cells	Membrane Staining intensity & pattern**	Score***
Up to 9% of cells	At most faint and discontinuous	0
≥ 10% of cells	At most faint & discontinuous	1+
≥ 10% of cells	Moderately intense continuous	2+
≥ 10% of cells	Intense continuous	3+

* **In cases of invasive carcinoma with an insitu component, only the invasive component was scored; because the biological behavior of any cancer (e.g. its metastatic potential etc.) is determined by its invasive portion.**

** **Intra-cytoplasmic staining was considered a non-specific reaction and was not included in the assessment of HER2/neu expression status.**

*** **To get reproducible statistical results, scores of (0 & 1+) were considered negative whilst scores of (2+ & 3+) positive.**

Fisher's exact test, Chi-square test & Mann-Whitney test were used for the statistical analysis of the variables. P Value ≤ 0.05 is considered statistically significant.

Results:

The overall mean age of the 38 cases of breast carcinomas was 47.0 years ± 8.3 years. Twenty four of the patients (63.2%) were below the age of 50 years (premenopausal). Twenty four cases (63.2%) were examples of IDC. The remaining 14 cases (36.8%) showed ILC. The former group comprised IDC (twelve cases, 31.5%); IDC + DCI of comedo type (seven cases; 18.4%) and IDC + PD (five cases; 13.2%). The 14 cases of ILC included were either ILC without in situ component (ten cases; 26.3% of the total) or ILC with LCI (four cases; 10.5% of the total). According to the Nottingham modification of Bloom- Richardson grading system one half (12 cases) of the ductal carcinomas were grade 2; the

remaining half were either grade 1 (four cases, 16.7%) or grade 3 (eight cases, 33.3%). More than two-thirds of the 38 cancers studied were within the size range of two to five cm in maximal dimensions (T2). Only about one fifth of the cases had a diameter below two cm (T1). Table 2

Table 2: The distribution of the patients with breast carcinoma by primary tumor size

Tumor size	No. of patients	Percentage
≤ 2 cm (T1)	7	18.4%
2- 5 cm (T2)	26	68.4%
> 5 cm (T3)	5	13.2%

There was an obvious deficiency in the harvest of axillary lymph nodes in a significant number of the mastectomy samples studied; accordingly we were obliged to simply divide the cases into those with axillary lymph node metastasis (N1) and those without (N0). The former group comprised almost two thirds of the cases (11 cases [64.7%] of the total 17 mastectomies). Of the 24 cases of IDC, HER-2/neu staining was positive in ten (41.7%) cases. None of the fourteen cases of ILC showed any degree of appreciable positive reaction. Because of that all they were dropped from further statistical analysis. (Fig. 1) The cells of all the DCI of comedo type showed HER-2/neu overexpression whereas the invasive components showed positive results in only three of the seven cases (42.9%). (Fig. 2) Statistical analysis revealed no significant differences in respect to HER2/neu expression between IDC with and without DCI (P= 0.23). All the five cases of IDC with overlying Paget disease showed positive staining for HER-2/neu overexpression. Both components; the cutaneous and the underlying IDC, showed cellular positivity. (Fig. 3) Four out of 24 cases of IDC were grade I, all of them were negative for HER-2/neu over-expression. (Fig. 4) Positive staining for the same marker was obtained, however, in five of the twelve grade II tumors (41.7%) and five of the eight of grade III tumors (62.5%). (Fig. 5) Statistical analysis revealed a significant difference between HER-2/neu overexpression and histological grade (P= 0.05) (table-3).

Table 3: HER-2/neu overexpression in relation to tumor grade in IDC

Tumor grade	HER-2/neu overexpression		Total
	+ve	-ve	
Grade I	0	4 (100%)	4
Grade II	5 (41.7%)	7 (58.3%)	12
Grade III	5 (62.5%)	3 (37.5%)	8
Total	10	14	24

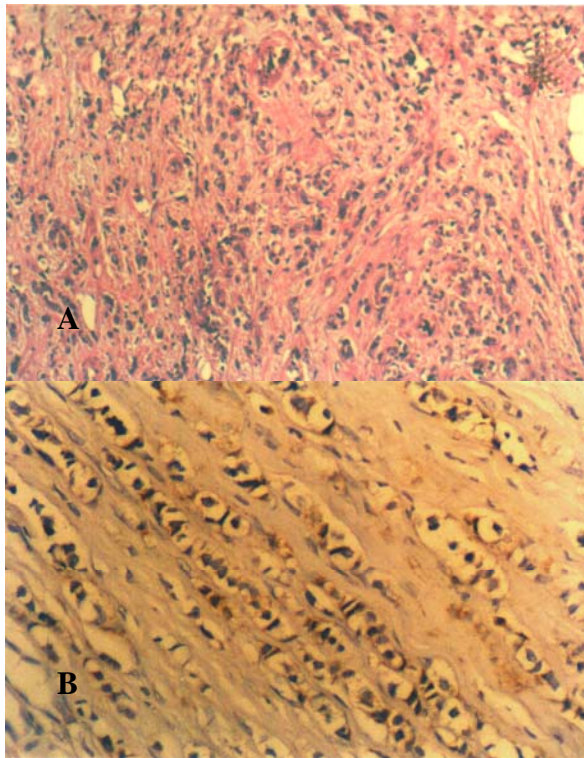


Figure 1: Classical infiltrative lobular carcinoma
A, a section showing small cells with typical Indian filing & targetoid arrangements of ILC (H& E stain; X40). **B**, a section from the same tumor; there is universally negative membrane staining of the tumor cells. The cytoplasmic brownish staining is nonspecific. (HER2/neu immunostain; X100)

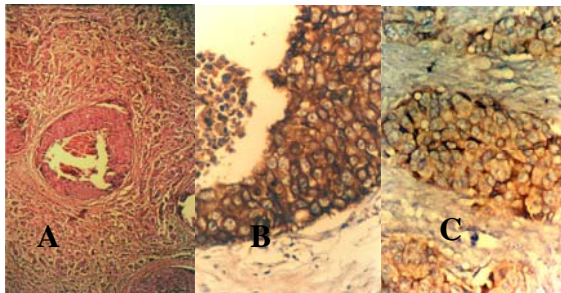


Figure 2. Infiltrative duct carcinoma with its in situ component of comedo type **A**, high-grade (poorly-differentiated) infiltrative duct carcinoma with its insitu component of comedo type. The latter shows extensive central necrosis (H&E X40). **B**, there is diffuse & strong membrane positivity of the insitu component (HER2/neu immunostain X400). **C**, trabeculae of malignant epithelial cells that represent the infiltrative component of ductal carcinoma; it shows weak & rather discontinuous positive staining (HER2/neu immunostain X400).

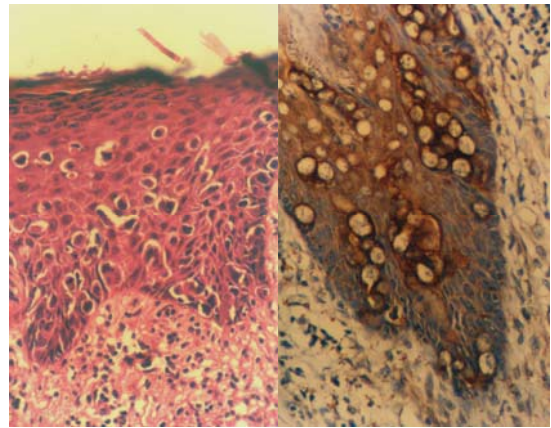


Fig. 3: Paget disease of the nipple Lt., H& E stained section of the nipple showing frankly malignant carcinoma cells insinuating between native epidermal cells. Rt., HER2/neu immunostain of the same case showing strong membrane positivity of the malignant cells.

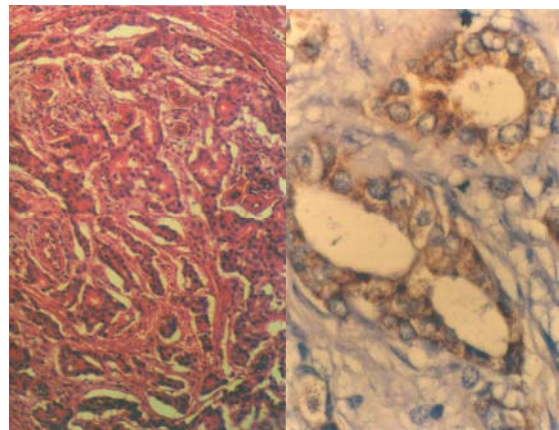


Fig.4: Infiltrative duct carcinoma, well-differentiated (grade 1) Lt., there is prominent tubular arrangement of the relatively small cells with monotonous nuclei that lack mitotic activity. Rt., HER2/neu immunostain showing absence of membrane staining. The cytoplasmic brownish granular staining seen is nonspecific.

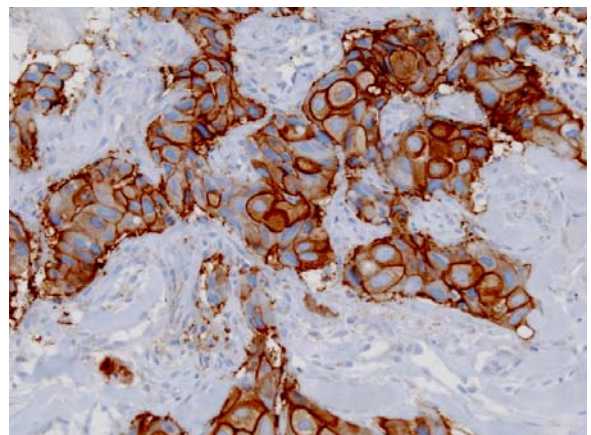


Fig. 5: infiltrative duct carcinoma Her2/neu immunostain. The malignant epithelial cells that form the invasive trabeculae of the cancer show diffuse strong membrane staining.

Discussion:

Breast cancer is a heterogeneous disease with variable clinical, pathological and biological characteristics. It has been found that breast cancer in many Asian and African countries tends to affect younger females, to present in advanced stage, and is associated with poor prognostic parameters.⁹ Thus; overall these cancers have a worse outcome in comparison with their counterparts in Western countries. ^{9, 10, 11, 12, 13, 14, 15} there is no doubt that the lack of public awareness of the seriousness of the disease may contribute to the advanced presentation. However, the biological aggressiveness in terms of poor differentiation, low steroid receptors expression and tendency to affect younger females remain unexplained. Abnormalities described in the structure & activity of several proto-oncogenes may contribute to the development or progression of breast cancer ¹⁶. The outcome of a lot of studies on the subject has been the proposal to regard HER2/neu as a marker of prognostic significance and of potential clinical utility ¹⁷. This study was done with the incentive that breast cancer in Iraq is not only the most common tumor in females, (about 14.3% of all malignant tumors) ¹⁸ but also these tumors display an aggressive natural history. Significant correlation between HER2/neu overexpression & histopathological type was reported in this study; in that 41.7% of all IDC cases were positive, whereas none of ILC was positive. (P=0.004). This negativity on the part of ILC was also the experience of Vijver et al & Poter et al in their separate studies. ^{19, 20} this high discrepancy between IDC & ILC regarding HER2/neu overexpression has led to the suggestion that there are differences between the two types at the molecular levels. Of the 63.2% of our patients who have had IDC, almost two thirds (41.7%), was HER2/neu positive. This result is in agreement with that reported by Khorshid et al & Aziz et al. who reported a positive frequency of 40% and 39.4% respectively. In contrast Al-Moundhir et al reported a much lower result of 19.4% of cases.^{21, 22, 23} these differences are probably related to differences in sample size, selection bias of the cases, and the use of commercially different kits. In this study the presence or absence of an in situ component of ductal carcinomas seems to have no statistically significant effect on HER-2/neu overexpression (P= 0.23). However, among cases of IDC with DCI of the comedo-type (seven cases); all the in situ carcinomas showed HER-2/neu overexpression whereas the invasive components showed positive results in only three cases (42.9%). Vijver et al ¹⁹ reported similar results. This discrepancy in the rate of frequency of positivity between the in situ and invasive components could be due to the fact that HER-2/neu overexpression might have been lost during tumor progression or alternatively, the activation of HER-2/neu was more likely to be important in tumor initiation than progression, being decreased as tumor pass from the in situ to invasive phase. the same author showed that among cases of

carcinoma in situ without invasive component, the ones with HER-2/neu overexpression showed a large-cell, comedo-type histological appearance in contradistinction to cases of small-cell, papillary or cribriform type, which were all negative. In this study, statistically significant correlation was found between HER-2/neu overexpression of invasive ductal carcinoma with overlying Paget's disease (P= 0.003). There was a complete agreement (100%) in HER-2/neu positivity of both the invasive ductal carcinoma and Paget's cells when both components were present simultaneously. Our findings in this context agree with that reported by Al-Moundhri et al and Vijver et al, in which HER-2/neu overexpression was detected in almost all cases of Paget's disease.^{19,23} The other significant correlation was observed between HER-2/neu overexpression and higher histological grades of invasive ductal carcinoma (P= 0.05). All cases of grade I invasive ductal carcinoma were negative for the marker, whereas 41.7% of grade II and 62.5% of grade III were positive. (Table 3) This is similar to the results of Khorshid et al & Aziz et al but disagree with Al- Moundhri et al, which revealed no statistical correlation between HER-2/neu overexpression and histological grading of the tumor.^{21, 22, 23} There seems to be no statistically significant correlation between the positivity of the marker and lymph node involvement (P= 0.36). This was not only our experience, but also of Khorshid et al, Al- Moundhri et al and Vijver et al.^{19, 21, 23} Depending on these and other studies, it has been concluded that lymph node involvement could be a prognostic rather than a selection criterion for adjuvant treatment.²⁴ Regarding other clinical parameters, no association was found between HER2/neu immunopositivity & patient age, menopausal state, vascular and/or lymphatic invasion & lymphocytic infiltration of the tumor. Similar results were reported by Aziz et al, Khorshid et al and Al- Moundhri et al studies. ^{21, 23} A strong correlation was found between HER-2/neu overexpression and the primary tumor size in our as well as Vijver et al study.¹⁹ This association between HER-2/neu overexpression and large tumor size may point to a larger replicative pool of these cancers which could be reflected as a higher growth rate. The main goal for studying HER-2/neu overexpression in invasive breast cancer is to select patients suitable for Herceptin therapy. Hopefully, in the near future HER-2/neu screening will become a routine test for all breast cancer patients. However, how many of the patients may potentially benefit from this novel, and currently costly therapy remains a big question for developing countries like Iraq to answer.

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