

Correlation of Her-2/Neu Expression with Conventional Clinicopathological prognostic Parameters in Breast Carcinoma

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ABSTRACT:

BACKGROUND:

The prognostic biomarkers in a disease provide information regarding outcome irrespective of therapy. Her-2/neu oncogene expression is an important prognostic and predictive marker for breast cancer. Her-2/neu expression can be determined by immunohistochemistry, which became a routine investigation to predict the response to tamoxifen therapy. There is evidence that over-expression of Her-2/neu is involved in breast cancer progression and there is suggestion that Her-2/neu over-expression plays a role in the early stages of breast tumorigenesis.

OBJECTIVE:

To evaluate Her-2/neu over-expression in breast carcinomas in the studied sample and to assess its immunohistochemical score status in term of accepted conventional clinicopathological parameters.

PATIENTS AND METHODS:

This is a descriptive study which was carried out from 2007-2012 on 64 breast carcinoma specimens. Paraffin embedded tissue of patients underwent modified radical mastectomy with axillary clearance were processed for Her-2/neu assessment from almost all breast patients in the province. Then the evaluation of the staining and assigning a score were performed which ranged from 0-3. The tumor grade was assessed using modified Bloom-Richardson grading system. Data on the patients age, the size of the tumor and lymph node status were obtained.

Statistical analysis were produced by the use of Chi square test and at the level of significant $\alpha < 0.05$ to compare the IHC results for HER-2 expression with the prognostic clinicopathological parameters.

RESULTS :

HER-2 over expression was seen in 23.5% (15/64) and the lymph nodes involvement status had a significant association with the immunohistochemical score for HER/2 oncogene and no significant association seen between HER-2 overexpression and other clinicopathological parameters including age, tumor size and tumor grade.

CONCLUSION :

The study reveals that the prevalence of HER-2 overexpression in breast cancers is less in our study compared with the western world and demonstrates association of the immunohistochemical score of HER-2/neu with some of the well-established pathological parameters of breast carcinoma, specially lymph node involvement. It justifies the use of the immunohistochemical score in assessing breast carcinoma and modified its treatment.

KEYWORDS: Breast carcinoma, Her-2, immunohistochemical score, Clinicopathological prognostic parameters

INTRODUCTION :

Breast cancer, the most terrifying cancer for female, is the commonest malignancy and the second leading cause of cancer death in women in Iraq and worldwide with several reports indicating a rising incidence. In Iraq, it has remained the commonest malignant tumor since the last three decades. Cases have increased

dramatically and constituted 14.3% of all types of cancers in 1997, the incidence is increasing in registered female cancers from 38% in 1976 to 47% in 1997 as documented by Iraqi cancer registry 1996 & 1999 and there is an increase in incidence in younger age groups; the peak frequency being recorded in the age group 40-49 years⁽¹⁾.

Breast cancer is a heterogeneous disease with variable biological and clinical characteristics.

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The racial influence in invasive breast cancer in terms of age at presentation, clinico-pathological features, and outcome of treatment has been widely reported. The descriptions of prognostic factors in breast cancer have exploded over the past several years. Pathologists have played a major role in identifying different histological and immunohistochemical markers that have a direct bearing on both the treatment and behavior of breast cancer⁽²⁾.

Besides several prognostic factors like tumor size, histological grade, steroid hormone receptor status, DNA ploidy, or lymph node status, the status of HER-2/neu serves as an additional parameter. HER-2/neu is involved in the regulation of normal cell growth and division and is expressed at low levels in many normal epithelial cells⁽³⁾.

HER-2 /neu (human epidermal growth factor receptor 2) ,proto-oncogene located on chromosome 17q21, encodes an 185KD membrane glycoprotein with intracellular tyrosine kinase activity after integration with its ligands, resulting in activation of diverse subcellular signal transduction pathways, thus HER-2 gene is critical in the control of growth, differentiation, and mobility of many normal and transformed epithelial cell types.⁽⁴⁾ Over expression of this gene has been associated with more aggressive disease and a poor clinical prognosis in 20-30% of patients with breast cancer and involved in breast cancer progression⁽⁵⁾ This hypothesis based on the high frequency of HER-2 overexpression among many invasive and non invasive breast disease⁽⁶⁾. This suggests that HER-2 play roles in early stages of breast carcinogenesis. Immunohistochemical evaluation of HER-2 gene overexpression represents a clinically attractive choice for evaluate the state of this proto-oncogene in malignant tumor cells because of its low cost, biological relevance, and technical availability in most pathology laboratories.

The aim of this study is to evaluate the immunohistochemical HER-2 overexpression and compare the score of HER-2 overexpression with other conventional common clinicopathologic parameters in a series of women with invasive ductal carcinoma of breast.

PATIENTS AND METHODS:

This is descriptive study was carried out from 2007-2012 on 64 breast carcinoma specimens at the oncology unite of Ramadi Teaching Hospital in Anbar governorate, west of Iraq. Paraffin

embedded blocks of breast carcinoma for patients underwent modified radical mastectomy with axillary clearance were processed for HER-2 assessment from almost all breast carcinoma patients in the Anbar governorate.

One 4-μ section from each submitted paraffin block was stained with hematoxylin and eosin to verify the presence of invasive ductal carcinoma and adequacy of fixation was sufficient for IHC analysis. From the selected blocks, 4-μ-thick sections were prepared and float mounted on adhesive coated glass slide for HER-2/neu staining (1:200 dilution for HER-2 –rabbit antihuman c-erb B2 oncoprotein antibody) Dako®. Best-preserved and best-stained areas of the sections were assessed. Then the evaluation of the staining and assigning a score were performed on the invasive component only. The criteria of positive reaction for HER-2 was assessed by scoring the proportion and intensity in 100 malignant cells performed at X 40 objective in 25 malignant fields of invasive ductal carcinoma. The scoring for the proportion of membrane staining pattern of malignant cells and the intensity of staining were taken in account and the staining being graded on the basis of a four point scoring system which ranged from 0 -3. The cytoplasmic immunostaining was noted but not incorporated into final scoring.

The tumor grade was asses on modified Bloom-Richardson grading system. Data on the patients age, the size of the tumor and lymph node status were obtained and recorded from the final histopatholgy reports of the patients with breast carcinoma (invasive ductal carcinoma).

Statistical analysis were produced by the use of Chi square test and at the level of significant alpha < 0.05 to compare the IHC results for HER-2 expression with the prognostic clinicopathological parameters.

A score for the proportion of stained cells (HER-2/neu reference value):

Negative: No staining is observed, or faint membrane staining present in <10% on infiltrating tumor cells (**Histoscor = 0**)

Negative: A faint /barely perceptible (incomplete) membrane staining in >10 % of infiltrating tumor cells (**Histoscor = 1**)

Weak positive: A weak-moderate complete membrane staining (equivocal) present in >10 % of infiltrating tumor cells (**Histoscor = 2**)

Strong positive: A strong complete membrane staining present in >10 % of infiltrating tumor cells (**Histoscor = 3**).

RESULTS:

The Age of the patients ranged from 26 to 75 years, with a mean of 46.5 years . The group of young women with breast cancer (<50years) included 31 patients (48.4%). The peak age frequency of 41-49 years was reported , which

included 43% of the breast cancer patients. Age was compared with the HER-2 scores to assess whether there is any difference between the breast cancers in young women and the rest (<50 years and ≥ 50 years) .(Table-1).

Table 1: The association of HER-2/neu scoring with the patient age .

HER-2 Scoring	Before 50		After 50		Total		P value
	No	%	No	%	No	%	
Negative=0	12	18.7	8	12.5	20	31.2	0.344
Negative=1	4	14.1	7	10.9	16	25	
Weak Positive =2	6	4.3	10	15.6	16	25	
Strong Positive=3	4	6.2	8	12.5	12	18.7	
Total	31	48.4	33	51.5	64	100	

Among the 36 negative HER-2 cases, 21 were younger than 50 years (58.3%) and 15 were older than 50 years (41.6%), Among the 28 positive HER-2 cases, 10 (35.7%) were younger than 50 years and 18 were older than 50 years (64.3%), these differences were statistically insignificant . $\chi^2 = 3.324$, df = 3 $P = 0.334$ (i.e. there is no

statistical significance correlation between patient age and HER-2/neu score) .

The **size of the tumor** was categorized into four groups (T1=<2cm, T2= 2-5cm , T3 >5cm ,T4 for those with chest wall extension , peau d'orange and ulceration)as it signifies different stages in the TNM Staging system. ⁽⁷⁾ (Table-2).

Table 2: The association of HER-2/neu with the size of the breast cancer.

Tumor size	0	1	2	3	Total	P value
T1	2	4	1	3	10 (15.6 %)	0.063
T2	13	22	2	2	39 (60.9 %)	
T3	2	5	3	2	12 (18.7 %)	
T4	0	1	1	1	3 (4.6 %)	
Total	17 (26.5%)	32(50%)	7(10.9%)	8(12.5 %)	64 (100%)	

The size of the tumor ranged from 1.2 to 9.5 cm, with a mean size of 3.4cm

The majority of patients 60.9%(39/64) had tumors measuring 2-5cm in the maximum dimension, the tumor measured >5cm in maximum diameter in 18.7 % (12/64) of the patients while the tumor size was <2cm in 15.6 % (10/64) of the patients. Three patients (4.6%) present with chest wall extension , peau d'orange and ulceration. $\chi^2 = 13.420$, df = 7 , $P = 0.063$ (i.e. there is no statistical significance correlation between tumor size and HER-2/neu score) .

The lymph node status was available for all 64 patients with mastectomy and axillary clearance . 18.7 % (12/64) was pNO as they did not have any lymph node metastasis. More than 81 % of our study patients have lymph node metastasis where 25% (16/64) were pN1 while 46.8% (30/64) were pN2 and 9.3 % (6/64) were pN3 (Table-3). ($\chi^2 = 14.216$, df = 7 , $P = 0.047$). (i.e. there is statistical significance association between lymph nodes states and HER-2/neu score) , the association is close the cut point of significant , may be due to the small number of cases in our study samples .

Table 3: The association of HER-2 scoring and level of lymph-node involvement.

HER-2 Scoring	Lymph nodes involvement				Total		P Value
	pN0	pN1	pN2	pN3	%	No	
Negative = 0	5(7.8%)	6(9.3%)	13(20.3%)	1(1.5%)	39	25	0.047
Negative = 1	4(6.2%)	6(9.3%)	12(18.7%)	2(3.1%)	37.5	24	
Weak Positive =2	1(1.5%)	3(4.6%)	2(3.1%)	1(1.5%)	10.9	7	
Strong positive =3	2(3.1%)	1(1.5%)	3(4.6%)	2(3.1%)	12.5	8	
Total	12	16	30	6	100	64	

The histological grade was assessed in all 64 cases. 35.9 % (23/64) was grade III tumors while 32.8 % (21/64) were grade II and 31.2% (20/64) were grade I , as shown in **(Table-4)**. For HER-2 score were increase in percentages of patients in grade III with strong positive staining(figure-1), but there was no statistical significant for this observation because the percentages are also high in grade I and II. Grade III tumors predominated accounting for 23 (35.9%) cases. These included six cases (26 %)showing HER-2 positivity and 17 cases (74%)

showing negative staining. Grade II tumors accounted for 21 (32.8%) cases. Four cases(19.1%) showed positive immune staining for HER-2(figure-2), while 17 cases (80.9 %)were negative. Twenty cases (31.2%) of grade I tumors were encountered, of which, Five cases (25 %) were HER-2 positive, while 15 cases (75 %) were negative. There was no statistically significant association between tumor grade and Her-2 overexpression status ($\chi^2 = 8.981$, $df = 7$, $P = 0.254$).

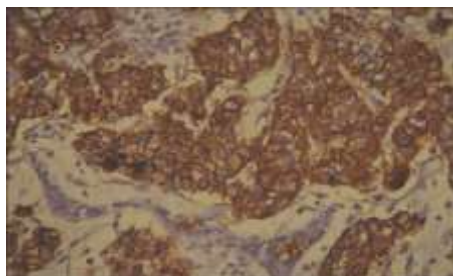


Figure 1 : Grade III invasive ductal carcinoma showing strong positive membrane IHC staining (Histo score = 3) for Her-2/neu. (Her-2/ neu +ve IHC stain, ×40).

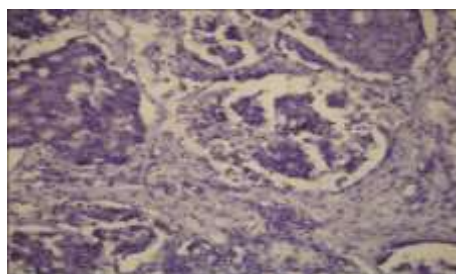


Figure 2 :Grade II invasive ductal carcinoma showing negative membrane IHC staining (Histo score=1) for Her-2/neu. (Her-2/neu IHC stain, ×40).

HER-2/NEU EXPRESSION

Table 4: The association of HER-2 scoring with the grade of the tumor.

HER-2 Scoring	Grade							Total	P value
	%	No	%	III	%	II	%		
Negative = 0	34.3	22	7.8	5	14	4	12.5	8	0.254
Negative = 1	42.1	27	18.7	12	12.5	8	10.4	7	
Weak positive=2	7.8	5	3.1	2	1.5	1	3.1	2	
Strong positive =3	15.6	10	6.2	4	4.5	3	4.6	3	
Total	100	64	35.9	23	32.8	21	31.2	20	

The immunohistochemical score for HER-2 was negative in 76.5% (49/64), where HER-2 was 0 in 31.2% (20/64) and was 1 in 45.3 % (29/64), indicating no HER-2 expression in the majority. About 12.5 % of the positive HER-2 cases (8 out

of 64) had strong positive stain (Histo score = 3) and 10.9% (7 out of 64) were weak positive HER-2 expression (Histo score = 2)(figure-3).

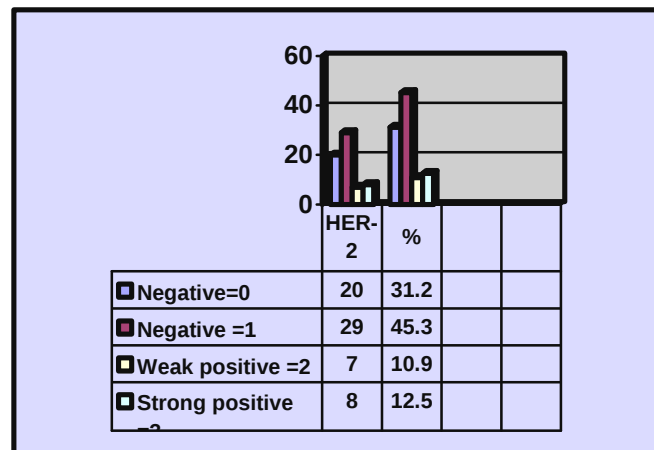


Figure 3 :HER-2 scoring in breast cancer .

DISCUSSION:

Breast carcinoma is a disease with a tremendous heterogeneity in its clinical behavior. Studies have shown that immunohistochemical determination of HER-2 positivity followed by trastuzumab administration is useful in the treatment of breast cancer. ⁽⁸⁾ .An expanded understanding of the biology of breast cancer has led to the identification of the Her-2/neu has been associated with aggressive biological behavior and poor clinical outcome .Following the original study by Salmon and coworkers in 1987 and many investigators have considered the prognostic potential of the HER-2 gene and protein in breast cancer ⁽⁹⁾ . Her-2 is over expressed in 20-30% of metastatic breast cancers. This over expression is associated with decreased survival and decreased relapse-free periods and so whose cancer shows HER-2/neu amplification

generally have a poor prognosis. Her-2 /neu gene amplification is also a predictive marker of responsiveness to selected forms of therapy. Clinical studies have demonstrated that alterations in HER-2 predict poor prognosis for breast cancer and are associated with features of tumor aggressiveness, such as absence of estrogen and progesterone receptors, high rate of cellular proliferation, advanced tumor stage, large tumor size, lymph node involvement and young age at diagnosis ⁽¹⁰⁾ The results of our study that carried out in Anbar province ,west of Iraq ,revealed that that 15 cases (23.5%) of 64 cases were HER-2 positive. Although there is a wide variation in HER-2 overexpression and amplification, our figure appears to be within the commonly accepted rate of 20% to 30% ⁽¹¹⁾ . It does appear similar to that reported in Jordan ⁽¹²⁾

however, to be lower than those reported in neighboring countries such as Lebanon ⁽¹³⁾ and Egypt ⁽¹⁴⁾. This is probably due to the high prevalence of high-grade tumors among our study population.

The ages of the breast cancer patients were ranging from 26 to 75 years, with a mean age of 46.5 years, 48.18 % of them were younger than 50 years . The peak age frequency of 41-49 years was reported in this study, which included 43 % of the breast cancer patients .Al-Alwan reported close results in a study carried out in Baghdad with a peak age frequency between 40-49 years⁽¹⁵⁾ .The mean age of Her-2/neu negative cases was 53 years with an age range 36 to 74 , while the mean age of Her-2/neu positive cases was 45 years . The mean age of Her-2 positive patients was 8 years less than those patients lacking Her-2 expression, a statistically no significant difference ($P = 0.344$). It should be pointed out that , the higher rates of Her-2 overexpression in young patients in our study have been documented in previous studies ⁽¹⁶⁾ . Same finding was reported in Adebamowo and coworkers study ⁽¹⁷⁾ . In contrast to our study , Ray et al study, reveals HER-2/neu over expression was significantly higher among post menopausal in comparison with pre-menopausal ⁽¹⁸⁾ . As an adverse prognostic factor, HER-2 positivity has been associated with resistance to certain types of chemotherapy. ⁽¹⁹⁾ . Finally, Although the mean age of Her-2/neu positive patients was 8 years less than those patients lacking HER-2 overexpression, the difference there was no significant association between Her-2/neu over expression and age which reflect hormonally state (menopausal status). Out of 64 patient included in our study 76.5 % were completely negative HER-2 and 23.5 % overexpress HER-2. It indicates that the majority of the breast carcinomas in the study sample have no HER-2 expression and would not respond to endocrine therapy . Regarding the cancer grade , 31.2% of cases were in grade I , 25 % of them show HER-2 overexpression , 32.8% of cases were in grade II , 19 % of them show HER-2 overexpression and 35.9% were in grade III, 26% of them show HER-2 overexpression , however, did not find a significant statistical association between tumor grade and the IHC detection of HER-2. In the present study, although there is no statistically significant deference , the overall grade of the tumor correlated well with the proportion of stained cells and the intensity of staining, the two components of the histoscore that justify the

assessment of the intensity of staining in giving a score .

In contrast to our results, some studies have indicated that there is generally more uniform loss of HER-2/neu over expression as the tumor becomes more anaplastic, In contrast, however, other reports showed that tumor grade has a prognostic effect of survival, which seemed to be indirect through the lymph node metastasis and not through the tumor grades , assuming that metastatic deposits tend to originate from the most poorly differentiated cells ⁽²⁰⁾ . this was supported by the non-significant association detected between HER-2 over expression and tumor grade.

It has been pointed to the high rate of malignant breast tumors in Iraq with poorly differentiated cells (aneuploid) and positive lymph node reported in 81.6% of Iraqi patients ⁽¹⁵⁾ .Al- Anbari 2009 reported higher percentages of patients in high grades (48% in grade II and 41% in grade III), and 50% of patients presented with tumors measuring less than 2 cm⁽²¹⁾ . Regarding the tumor size relatively large percentage (60.9%) of breast cancer patients presented with T2 . Our results show a tendency of HER-2 overexpression to be more associated with larger tumor size ,where tumors expressing HER-2 were on the average 1.6 cm larger than those lacking HER-2 expression, although this difference was not statistically significant , the fraction of larger tumors (T3 & T4) tended to have higher rates of HER-2 expression than those of T2 (46.7 % versus 10.2%), but this difference was not statistically significant ($P = 0.063$). In contrast in Egypt, clinical T1, T2, T3 and T4 lesions were 1.2%, 30.0%, 26.4% and 42.4%, respectively. The mean tumor size was 4.5 cm ⁽²²⁾ ,while Others studies data indicating the association of HER-2 positively with large tumors⁽²³⁾

Regarding the association of HER-2 overexpression with lymph node involvement ,our study reveal that 80 % (12/15) of HER-2 overexpressing tumors had lymph node metastases and 81 % (40/49) of HER-2 negative cases had lymph node metastasis , ($P = 0.047$). this difference was statistically significant. Although the association is close the cut point of significance ,we believe that future studies with larger numbers of patients are needed to confirm statistically significant association of Her-2/neu expression with nodal metastases .Other studies have shown similar result to our patients where they confirmed a direct relationship between lymph node metastases and Her-2 expression ⁽²⁴⁾

meanwhile; Sutterlin et al ,2000 reported that there was no significant relation between HER-2 over expression and lymph node involvement ⁽²⁵⁾ Korkolis et al, added in their study that HER-2 overexpression might characterize a subgroup of node negative patients with poor prognosis who could benefit from an aggressive adjuvant therapy ⁽²⁶⁾.

The majority of patients included in the present study had lymph node metastasis by the time they sought treatment. The majority (64.2%) presented with T2 (tumor size between 2 and 5cm) . Hence, the majority do not present in the early stage. This may be due to the absence or inefficient breast screening program in our governorate.

CONCLUSION:

The prevalence of HER-2/neu overexpression in breast cancers is less in our study compared with the western world ..

Her-2/neu overexpression was correlated with some unfavorable clinico-pathological variables of breast carcinoma , specially for lymph nodes status .

It justifies the use of HER-2 /neu immunohistochemicalscore in assessing the breast cancers and modified the treatment accordingly .

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