# Over expression of HER2/ neu oncogene in breast cancer in Diwanya city by immunohistochemistry

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**ألخلاصه:-**

يهدف البحث إلى دراسة التعبير المناعي النسيجي الكيميائي لجين أل HER-2/neu كمؤشر للتغيرات الجينية الطارئة في ألخلايا السرطانية لأورام ألثدي ولمعرفة ترابط هذا التغير مع ثوابت أخرى مثل عمر المريض و درجة تمايز الورم ونوعه. تمت دراسة ٤٩ عينة من عقدة الثدي في مختبرات خاصة للفترة منذ بداية كانون الثاني ٢٠٠٨ وحتى تشرين الأول ٢٠٠٩ ، تراوحت أعمارهم بي٢٠ و ٧٣ سنه مع معدل عمر ٤٦,٥ سنه. أظهرت الدراسة المناعية النسيجية أن تعبير أل- HER-2/neu كان موجبا في٣٢,٢٥% من سرطان الثدي حيث كان تعبير ال- HER-2/neu أكثر في المرضى ضمن مجموعة العمر الاكبر من خمسين عاما , وفي الأورام العقدية عنه في الأورام الفصية و في ألأورام ذات التمايز المتوسط (الدرجة الثانية ). مع عدم وجود ترابط بين ألتعبير المناعي النسيجي ا ل- HER-2/neu مع العمر (قيمة ألفا > ٠.٠٥) كما انه توجد علاقة واضحة لتعبير ا ل- HER-2/neu مع نوع الورم و درجة التمايز (قيمة ألفا < ٠.٠٥). مما يدل على أن جين أل-HER-2/neu يلعب دور أساسي في تقييم حالة سرطان الثدي المستقبلية وانتشاره.

**Abstract :-** This study was conducted to estimate the over expression of HER-2\neu protein in human breast carcinoma and to show its possible correlation to the pathological parameters (histological type, grade and age). We evaluated the available tissue blocks of 49 patients with breast lesions who had collected from private laboratory between January 2007 and October 2009. All cases were female. The mean age of the patients was 46.5 years (range, 20 to 73 years old). 38(77.5%) cases were of infiltrative ductal carcinoma and 11(22.5%) cases were of infiltrative lobular carcinoma , The tumors were grade I, II, and III in 3(7.9%),24(63.2%),and11(28.9) cases respectively.

From 49 cases, A total of 16 (32.25%) cases were positive for overexpression of *HER-2/neu* oncogene , all the positive cases was with IDC , the intermediate histological grades of the breast IDC were associated with highest expression of *HER-2/neu*. There were 14 cases of *HER-2/neu*-positive (58.4%) with a grade II tumor, 2 (37.5%) with grade III .There was a significant relation in HER-2/neu overexpression with the invasive ductal carcinoma and grades of tumor but no correlation was detected between HER-2/neu overexpression and the patient's age.

**Aim of the Study:** This study was conducted to estimate the overexpression of HER-2\neu protein in human breast carcinoma and to show its possible correlation to the pathological parameters ( types , grade and age) .

***Key ward :- breast IDC , HER-2/neu oncogene , IHC***

## Introduction :

According to Iraqi Cancer Registry, breast carcinoma is the most frequent cancer among women. It forms 14.3% of all malignant tumors and 30% of the registered female cancers with the sharp increase in incidence of this tumor in young age group. The average age of patients with breast carcinoma in Iraqi females is 45 years [1,2] **.** Breast cancer is strongly related to age with only 5% of all breast cancers occur in women under 40 years old[3]. Approximately 30 percent of breast cancers have an amplification of the *HER2/neu* gene or overexpression of its protein product.[4] Overexpression of this receptor in breast cancer is associated with increased disease recurrence and worse prognosis. Because of its prognostic role as well as its ability to predict response to trastuzumab (Herceptin US brand name) , breast tumors are routinely checked for overexpression of HER2/neu. Overexpression also occurs in other cancer such as

ovarian cancer, stomach cancer, and biologically aggressive forms of uterine cancer, such as uterine serous endometrial carcinoma.[5]

**HER2/neu** (also known as **ErbB-2**) stands for "Human Epidermal growth factor Receptor 2" and is a protein giving higher aggressiveness in breast cancers. It is a member of the ErbB protein family, more commonly known as the epidermal growth factor receptor family. HER2/neu has also been designated as **CD340** (cluster of differentiation 340) and **p185**. It is encoded by the *ERBB2* gene[6].

HER2 is a cell membrane surface-bound receptor tyrosine kinase and is normally involved in the signal transduction pathways leading to cell growth and differentiation. It is encoded within the genome by HER2/neu, a known proto-oncogene. HER2 is thought to be an orphan receptor, with none of the EGF family of ligands able to activate it. However(5), ErbB receptors dimerise on ligand binding, and HER2 is the preferential dimerisation partner of other members of the ErbB family.[6,7] The *HER2* gene is a proto-oncogene located at the long arm of human chromosome 17(17q21-q22).[8]

**Material and Method :-**

We evaluated the available tissue blocks of forty nine patients with breast lesions who had collected from private laboratory between January 2008 and October 2009. Three micrometer thick sections were prepared from paraffin-embedded tissue blocks and stained by hematoxylin-eosin method. Tumor grade was then determined using the World Health Organization. A manual avidine-biotin-peroxidase complex procedure was used in the immunohistochemical analysis (DakoCytomation, Copenhagen, Denmark); The membrane staining intensity and pattern of HER-2/neu expression were considered for scoring according the breast cancer HER-2/neu scoring system [9] . table [1]. Data were analyzed using the SPSS software and the chi-square was used .

**Table [1] :-HER-2/neu scoring system**

| Score | HER-2/neu protein overexpression | Membrane staining pattern |
|---|---|---|
| 0 | Negative | No membrane staining is observed or membrane staining is observed in less than 10% of tumor cells |
| +1 | Positive | A faint barely perceptible membrane staining is detected in more than 10% of tumor cells. The cells are only stained in part of their membrane |
| +2 | Weak positive | A weak to moderately intense complete membrane staining is seen in more than 10% of cells |
| +3 | Strong positive | A strong complete membrane staining is observed in more than 10% of tumor cells |

**Result:-**

All cases in our study were female. The mean age of the patients was 46.5 years (range, 20 to 73 years old ). 38(77.5%) cases were with invasive ductal carcinoma and 11(22.5%) cases with invasive lobular carcinoma . From 49 cases of breast cancer , A total of 16 (32.25%) patients were positive for overexpression of *HER- 2/neu* oncogene and all cases was infiltrative ductal carcinoma only, There was a statistical significant difference in HER-2/neu overexpression with the invasive ductal carcinoma [table 2]. The positive malignant cases was with strong positive 3+ in 26.3% [figure 2] and in score 1 and 2 in 7.8% [figure 3 and 4 ], [table 3] . The invasive ductal carcinoma cases were graded as grade I, II, and III in 3(7.9%), 24 (63.2%), and 11 (28.9%) cases, respectively. intermediat histological grades of the breast IDC were associated with highest expression of *HER-2/neu*. There were 14 cases of *HER-2/neu*-positive (58.4%) with a grade II tumor, 2 (37.5**%**) with grade III, and there is no expression in grade I with statistical significant relation ship between expression of her2/neu and grades of tumor ($P < 0.05$) [table 3] also the expression was slightly higher in the older age group and there was no statistical correlation between HER-2/neu overexpression and the patient's age. [table 4]

**Table 2:- HER-2/neu over expression between the two histopathological types.**

| | | | Positive | Negative | Total |
|---|---|---|---|---|---|
| Types of tissue | IDC | No. of cases | 16 (41.7%) | 22(58.3%) | 38(77.5%) |
| | ILC | No. of cases | 0 | 11(100%) | 11(22.5%) |
| Total | No. of cases | | 16(32.3%) | 33 (67.7%) | 49 (100%) |
| P value =0.0086 | | | | | |

**Table 3 :-HER2/neu expression in correlation of scoring system.**

| Scores | Invasive ductal carcinoma | Invasive lobular carcinoma |
|---|---|---|
| 0 | 22 (57.8%) | 11(100) |
| 1+ | 3(7.8%) | 0 |
| 2+ | 3(7.8%) | 0 |
| 3+ | 10(26.3%) | 0 |
| Total | 38(100%) | 11(100%) |

**Table3:-HER-2/neu overexpression in relation to tumor grade of invasive ductal carcinoma.**

| Grades | | Positive | Negative | Total | |
|---|---|---|---|---|---|
| Grade 1 | Number of cases | 0 | 3(100%) | 3( 7.9%) | |
| Grade 2 | Number of cases | 14(58.4%) | 10(41.6%) | 24(63.2%) | P value = 0.0251 |
| Grade 3 | Number of cases | 2(37.5%) | 9(62.5%) | 11(28.9%) | |
| Total | Number of cases | 16(32.25%) | 22(57.8%) | 38(100%) | |

**Table 4 :- The age of the malignant cases range from 27-75 years ,with overall mean age was 55 years old.**

| | | Positive | Negative | total | |
|---|---|---|---|---|---|
| Age group | 20-45 | 5(25%) | 13(75%) | 18(36.7%) | |
| | 46-73 | 11 (26.4%) | 20 (73.6%) | 31(63.3%) | P value >0.05 |
| Total | No. of cases | 16 (23.25%) | 23 (76.75%) | 49(100%) | |

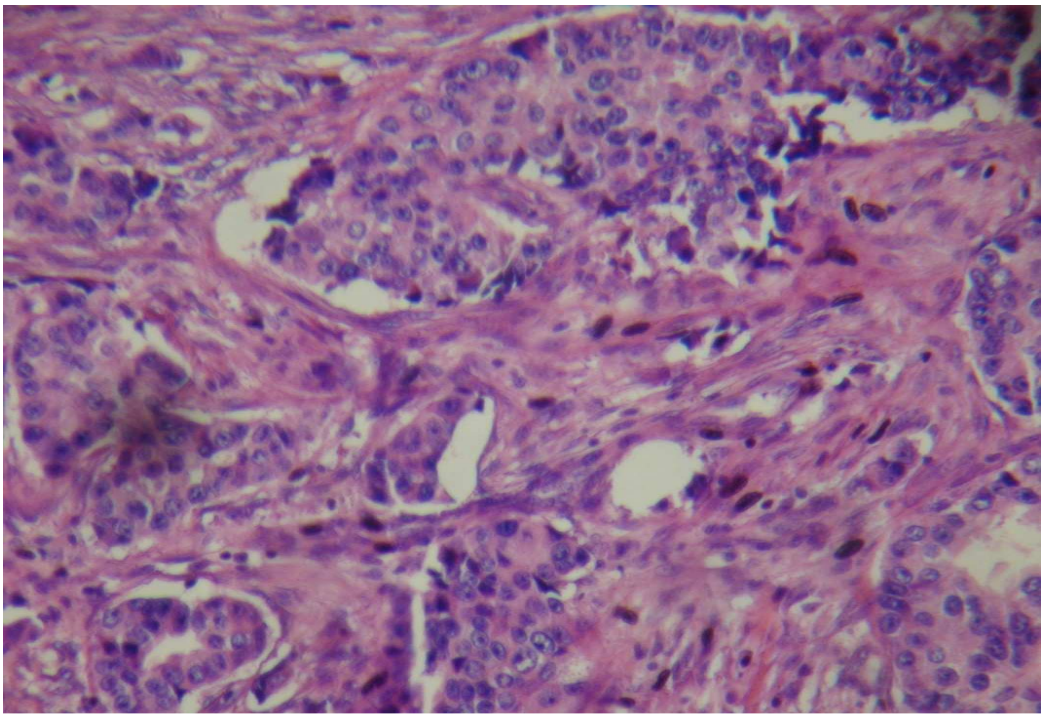

**Figure 1:- score zero of HER-2/neu expression in breast carcinoma**

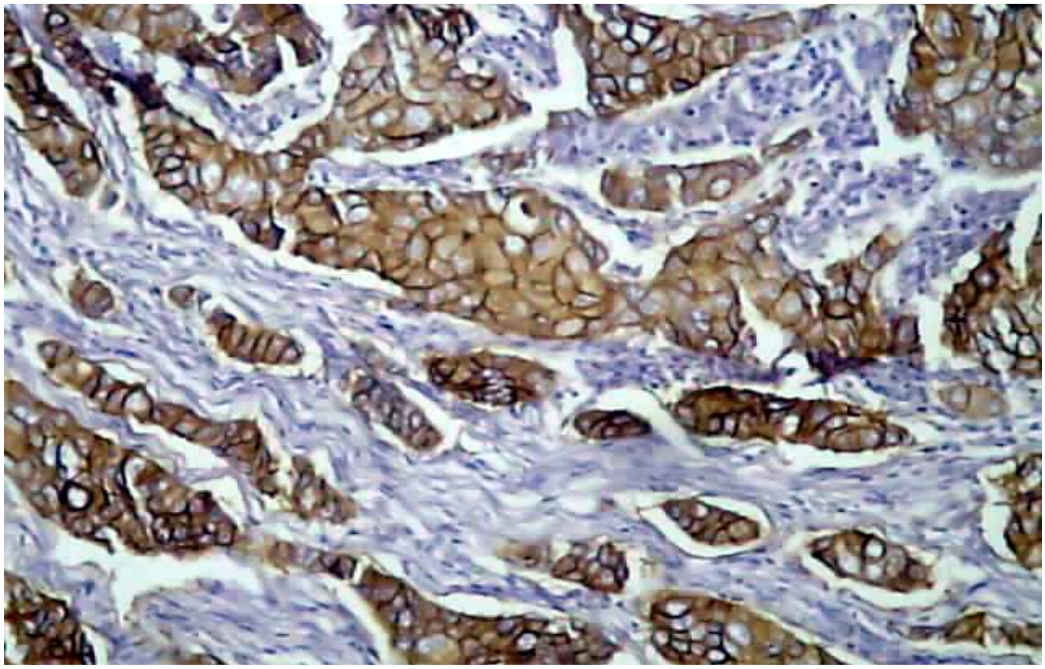

**Figure 2:- score 3 of HER-2/neu expression in grade II IDC.**

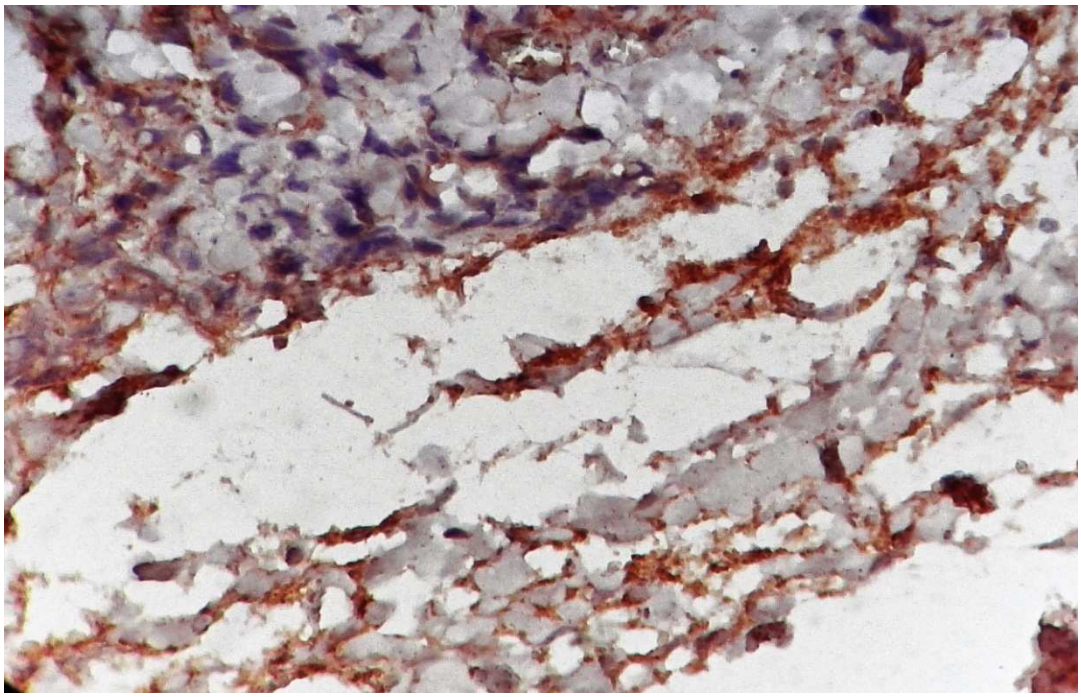

**Figure 3:- score 1of HER-2/neu expression in grade III IDC.**

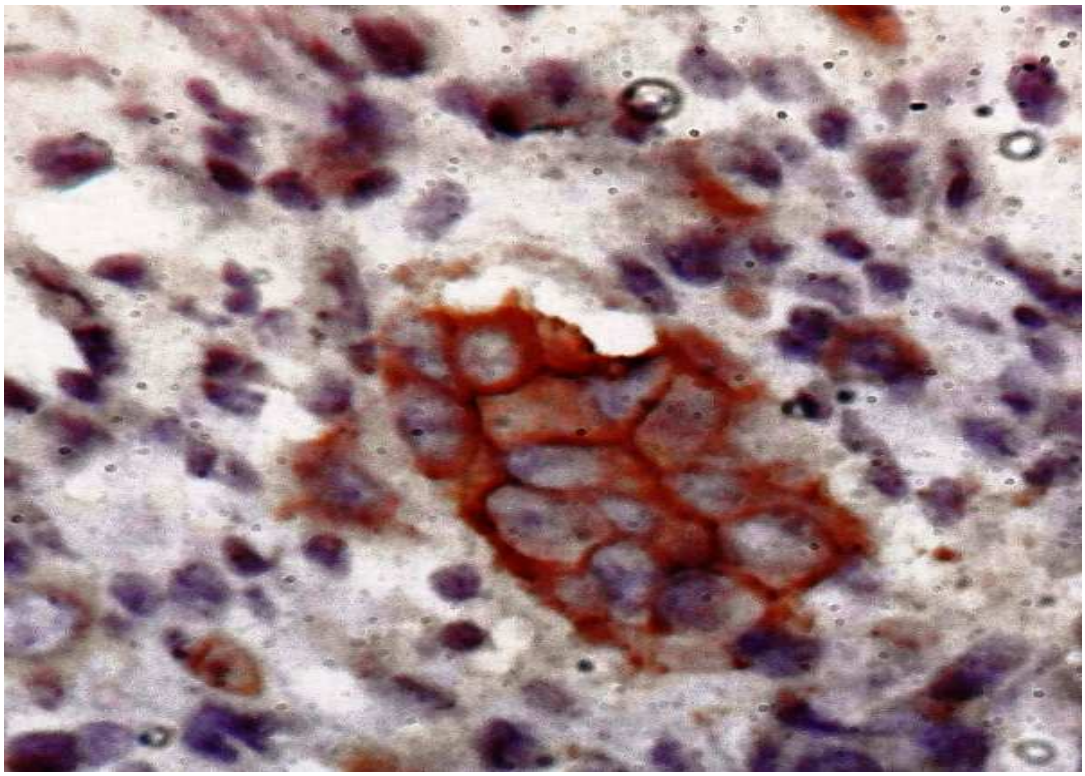

**Figure 4:- score 1of HER-2/neu expression in grade II IDC.**

**Discussion:-**

Our results have clarified that (32.25%) of the breast cancer cases were expressing HER2/neu and all the positive expressed cases were infiltrative ductal carcinoma with significant relationship between HER2/neu expression and type of breast cancer ; this in agreement with the Lobna Ayadi et al 2008[10] and Maurie Markman et al 2009 [11]. Also our study is in agreement with the study by Owens MA et al 2004[12] , 59% were positive on IHC and 23.6% were amplified on FISH however, the percentage of positive cells for *HER-2/neu* was greater in invasive tumors suggesting that this gene can be used in determination of the prognosis of breast cancer.

According to the present study , intermediat grade of the breast carcinoma is accompanied by *HER-2/neu* overexpression, Our results have clarified that her2/neu expression was slightly higher in grade II than grade III with significant correlation with grade of breast cancer cases; this in agreement with the Maurie Markman et al 2009 [11], Marjan Rahnamaye et al 2009 in spit of that they taking more cases in grade III[13] and Lobna Ayadi et al[10], where a significant relation was detected in the expression of this protooncogene and grade of the tumors; their method was more sensitive since they used fluorescence in situ hybridization than immunohistochemistry. In present study patients age ranges from (20-73) years with a mean of 46.5 years, and more than 50% of patient was above the age of 50 years old with no significant relationship between the expression of her2/neu and the age of the patient which is agree with Lobna Ayadi et al 2008[10] where the mean age was 51year.

**Conclusion:-**

Since most previous studies have shown a relationship between the tumor grade and expression of HER-2/neu oncoprotein, as we did in the present study, this gene can be used in determination of the prognosis of breast carcinoma . Comprehensive research with longer follow-up period and larger sample sizes are needed for further elucidation of the role of the oncogenes.

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