

## Immunohistochemical study of HER-2 /neu in invasive ductal carcinoma of breast

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### الخلاصة

يهدف البحث إلى دراسة التعبير المناعي النسيجي الكيميائي لجين أَل HER-2/neu كمؤشر للتغيرات الجينية الطارئة في خلايا السرطانية لأورام الثدي ومقارنته في النسيج الحميد ولمعرفة ترابط هذا التغير مع ثوابت أخرى مثل عمر المريض ودرجة تمايز الورم. تمت دراسة 50 عينة من عقدة الثدي في مختبرات مستشفى الديوانية التعليمي للفترة منذ بداية كانون الثاني 2007 وحتى كانون الأول 2010 ، تم جمع 38 عينة من مرضى مصابين بسرطان الثدي و 12 عينة من ورم الثدي الحميد ، تراوحت أعمارهم بين 33 و 70 سنة مع معدل عمر 55 سنة ، وقد قورنت المجموعتان مع بعض. أظهرت الدراسة المناعية النسيجية أن تعبير أَل- HER-2/neu كان موجبا في 31.5% من سرطان الثدي، ولا يوجد تعبير في عينات الورم الحميد. كان تعبير أَل- HER-2/neu أكثر في المرضى ضمن مجموعة العمر الأكبر من خمسين عاما ، في الأورام ذات التمايز الضعيف (الدرجة الثالثة ) عنه في الأورام ذات التمايز القوي والمتوسط (الدرجة الأولى والثانية). مع عدم وجود ترابط بين التعبير المناعي النسيجي أَل- HER-2/neu مع العمر (قيمة ألفا < 0.05) كما انه توجد علاقة واضحة لتعبير أَل- HER-2/neu مع درجة التمايز ( قيمة ألفا > 0.05). مما يدل على ان جين أَل-HER-2/neu يلعب دور أساسي في تقييم حالة سرطان الثدي المستقبلية وانتشاره وتحديد أهمية الاستجابة للعلاج .

### Abstract

This study was conducted to estimate the overexpression of HER-2\neu protein in human breast carcinoma in comparison to benign breast lesions and to show its possible correlation to the pathological parameters (histological type, grade and age). We evaluated the available tissue blocks of 50 patients with breast lesions (38 with invasive ductal carcinoma & 5 cases with fibroadenoma , 5 cases with fibrocystic disease & 2 cases with normal tissue) who had referred to Al-diwaniya Hospital between January 2007 and December 2010 All cases were female.

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The mean age of the patients was 55 years (range, 33 to 77 years). The tumors were grade 1, 2, and 3 in 6 (15.7%), 19 (50%), and 13 (34.3%) cases, respectively. From 38 cases of IDC, a total of 12 (31.5%) patients were positive for overexpression of *HER-2/neu* oncogene and from 12 cases of non-malignant cases all of them were negative for overexpression of *HER-2/neu* oncogene. The positive cases were with strong positive 3+ in 21.2% and in score 1 and 2 in 5.2%. High histologic grades of the breast IDC were associated with increased expression of *HER-2/neu*. There were 2 cases of *HER-2/neu*-positive (16.6%) with a grade 1 tumor, 4 (33.4%) with grade 2, and (50%) with grade 3 ( $P = .002$ ). There was a significant difference in *HER-2/neu* overexpression with the invasive ductal carcinoma and 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 in comparison to benign breast lesions and to show its possible correlation to the pathological parameters (grade and age).

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

## Introduction

Worldwide, breast cancer is the most common cancer in women, after skin cancer, representing 16% of all female cancers.<sup>[1]</sup> Mortality worldwide is 25% greater than that of lung cancer in women.<sup>[2]</sup> In 2004, breast cancer caused 519,000 deaths worldwide (7% of cancer deaths; almost 1% of all deaths).<sup>[3]</sup> The number of cases worldwide has significantly increased since the 1970, a phenomenon partly attributed to the modern lifestyles.<sup>[4][5]</sup>

The incidence of breast cancer varies greatly around the world: it is lowest in less-developed countries and greatest in the more-developed countries. In the twelve world regions, the annual age-standardized [incidence rates](#) per 100,000 women are as follows: in Eastern Asia, 18; South Central Asia, 22; sub-Saharan Africa, 22; South-Eastern Asia, 26; North Africa and Western Asia, 28; South and Central America, 42; Eastern Europe, 49; Southern Europe, 56; Northern Europe, 73; Oceania, 74; Western Europe, 78; and in

North America, 90.<sup>[6]</sup> 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 <sup>[7,8]</sup>. Breast cancer is strongly related to age with only 5% of all breast cancers occur in women under 40 years old.<sup>[9]</sup> 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<sup>[10]</sup>. 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<sup>(11)</sup>, ErbB receptors dimerise on ligand binding, and HER2 is the preferential dimerisation partner of other members of the ErbB family.<sup>[10,11]</sup> The *HER2* gene is a proto-oncogene located at the long arm of human chromosome 17(17q21-q22).<sup>[12]</sup> HER2 and cancer Approximately 30 percent of breast cancers have an amplification of the *HER2/neu* gene or overexpression of its protein product.<sup>[13]</sup> 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.<sup>[14]</sup> The oncogene *neu* is so-named because it was derived from a rodent glioblastoma cell line, which is a type of neural tumor, hence 'neu.' HER2 is named because it has a similar structure to human epidermal growth factor receptor, or

HER1. ErbB2 was named for its similarity to ErbB (avian erythroblastosis oncogene B), the oncogene later found to code for EGFR. Gene cloning showed that *neu*, HER2, and ErbB2 are the same. HER2 is co-localized, and, thus, most of the time, co-amplified with the gene GRB7, which is also a proto-oncogene (active in, e.g., breast cancer, testicular germ cell tumor, gastric cancer, and esophageal cancer). It is revealed that patients with ER+/HER2+ compared with ER-/HER2+ breast cancers may actually benefit more from drugs that inhibit the PI3K/AKT molecular pathway.<sup>[5]</sup>

### **Testing Methods of HER-2/neu**

The two main methods used for HER2 testing are immunohistochemistry (IHC) and fluorescence in-situ hybridization (FISH).<sup>[15,16]</sup>

**Immunohistochemistry (IHC):-** Immunohistochemistry (IHC) can show how much of the HER2 protein is present in the tumor sample. The HER2 level is graded from 0 to 3+.

0–1+ means that a normal amount of the HER2 protein is present and the result is HER2-negative

- 2+ means that a moderate amount of the HER2 protein is present
- 3+ means that there is a higher than normal level of HER2 protein and the result is HER2-positive. When a tumour is scored at 2+, UK testing guidelines recommend that a further test is carried out. This is because a result of 2+ does not always mean a cancer cell has a high level of HER2. In this situation, an extra test (FISH) is used to give a definite result.

### **Fluorescence in-situ hybridization (FISH)**

*Whereas IHC measures the level of HER2 protein in the tumour sample, FISH testing measures the amount of the HER2/neu gene in each cell. This gene is responsible for the overproduction of the HER2 protein.*

There is no number scale for FISH testing The result is either:

- FISH-negative – normal levels of the gene are present, or
- FISH-positive – excessive amounts of the gene are present. This is sometimes called gene amplification.

**Material and Method**

We evaluated the available tissue blocks of 50 patients with breast lesions (38 with invasive ductal carcinoma & 5 cases with fibroadenoma , 5 cases with fibrocystic disease & 2 cases with normal tissue) who had referred to Al-diwaniya Hospital between January 2007 and December 2010. 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 <sup>[17]</sup> . 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

## Results

All cases in our study were female. The mean age of the patients was 55 years (range, 33 to 77 years). The tumors were grade 1, 2, and 3 in 6 (15.7%), 19 (50%), and 13 (34.3%) cases, respectively. From 38 cases of IDC, a total of 12 (31.5%) patients were positive for overexpression of *HER-2/neu* oncogene [table 2] and all 12 cases of non malignant cases was negative for overexpression of *HER-2/neu* oncogene [table 2]. The positive malignant cases was with strong positive 3+ in 21.2% [figure 4] and in score 1 and 2 in 5.2% [figure 2 and 3], [table 3]. High histologic grades of the breast IDC were associated with increased expression of *HER-2/neu*. There were 2 cases of *HER-2/neu*-positive (16.6%) with a grade 1 tumor, 4 (33.4%) with grade 2, and (50%) with grade 3 with statistical significant relationship ( $P = .002$ ) [table 4]. There was a statistical significant difference in *HER-2/neu* overexpression with the invasive ductal carcinoma and No correlation was detected between *HER-2/neu* overexpression and the patient's age. [table 5]

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

			Positive	Negative	Total
Types of tissue	IDC	No. of cases	12(31.5%)	26 (68.5%)	38 (76%)
	Non malignant	No. of cases	0	12 (100%)	12 (12%)
Total	No. of cases		12 (24%)	38 (76%)	50 (100%)
P value <0.05					

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

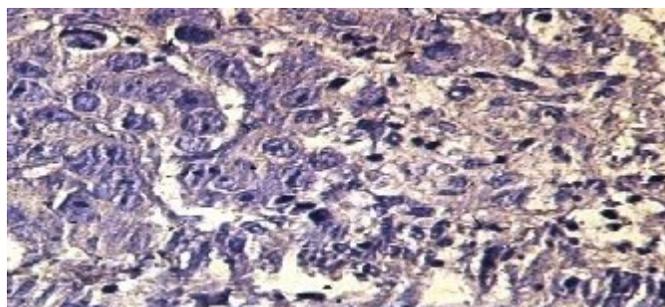
Scores	Invasive ductal carcinoma	Benign tissue	Total
0	26 (68.4%)	12(100)	38(76%)
1+	2(5.2%)	0	2(4%)
2+	2(5.2%)	0	2(4%)
3+	8(21.2%)	0	8(16%)
Total	38(100%)	12(100%)	50(100%)

**Table4:-HER-2/neu overexpression in relation to tumor grade of invasive ductal carcinoma.**

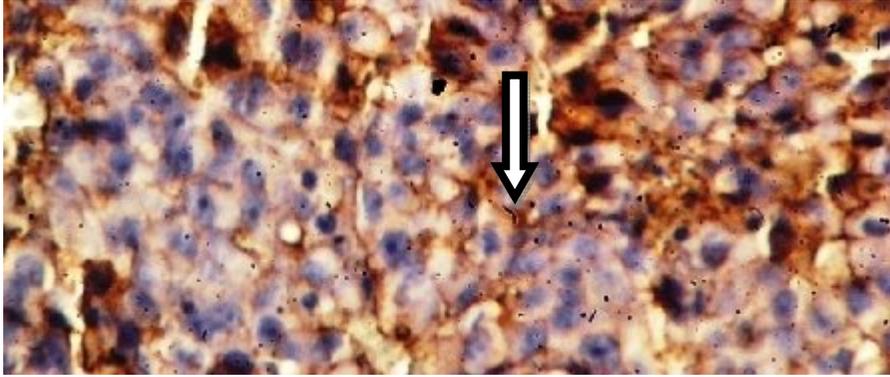
Grades		Positive	Negative	Total	P value <0.05
Grade 1	Number of cases	2(16.6%)	4(15.3%)	6(15.7%)	
Grade 2	Number of cases	4(33.3%)	15(57.6%)	19(50%)	
Grade 3	Number of cases	6(50%)	7(26.9%)	13(34.3%)	
Total	Number of cases	12(31.5%)	26(68.5%)	38(100%)	

**Table 5 :- The age of the malignant cases range from 33-77 years ,with overall mean age was 55 & SD 14.06.**

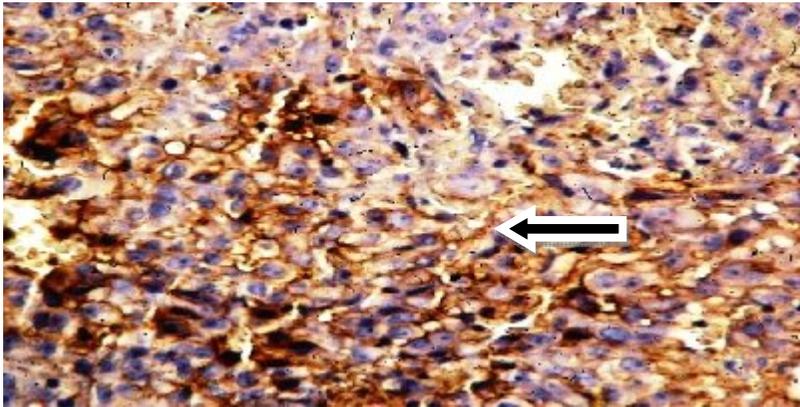
			Positive	Negative	total
Age group	33-39	No. of cases	1 (8.4%)	5 (19.2%)	6 (15.7%)
	40-49	No. of cases	4 (33.4%)	5 (19.2%)	9 (23.6%)
	50-59	No. of cases	3 (25%)	5 (19.2%)	8 (21%)
	60-69	No. of cases	2 (16.6%)	7 (27%)	9 (23.6%)
	70-79	No. of cases	2 (16.6%)	4 (15.4%)	6 (15.7%)
total	No. of cases		12 (31.5%)	26 (68.5%)	38 (100%)



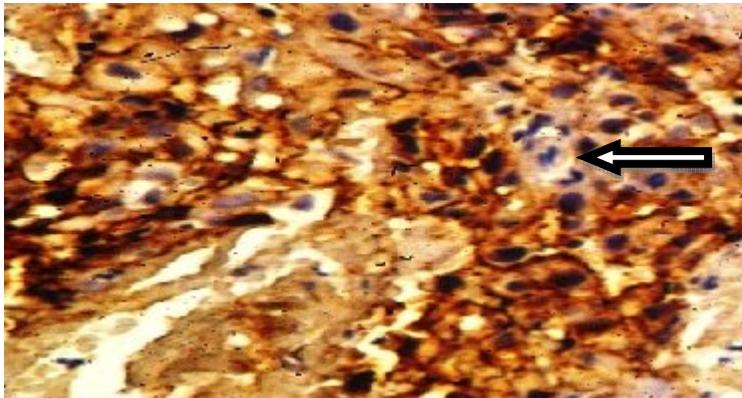
**Figure 1:- Score zero of HER-2/neu expression in breast carcinoma**



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



**Figure 3:- Score +2 of HER-2/neu overexpression in grade III IDC .**



**Figure 4:- Score +3 of HER-2/neu over expression in grade III IDC .**

## Discussion

According to the present study , higher grade of the breast carcinoma is accompanied by *HER-2/neu* overexpression. In the study by Lobna Ayadi et al<sup>[18]</sup> , Correlation of HER-2 overexpression with clinico-pathological parameters in Tunisian breast carcinoma in 155 patients with breast carcinoma , there is a significant relation was detected in the expression of this protooncogene and grade of the tumors;, but their method was more sensitive since they used fluorescence in situ hybridization than immunohistochemistry. In present study patients age ranges from (33-77) years with a mean of 55, and 50% of patient was above the age of 50 years which is agree with Lobna Ayadi et al 2008 where the mean age was 51years.

Most our cases were in age group  $\geq 50$  years old; this was in disagreement with this study<sup>(18)</sup> . Our results have clarified that (32.3%) of the breast cancer cases were expressing HER2/neu in cell membrane and cytoplasmic stain in their histological section. HER-2 was overexpressed (3+) in 21.2% of the cases. With significant relationship between HER2/neu expression and grade of breast cancer cases which are distributed as 16.6% in grade I ;33.3% in grade II & 50% in grade III ; this in agreement with the Maurie Markman et al 2009 [30%]<sup>(18)</sup> and higher than Lobna Ayadi et al 2008 [26%]<sup>(19)</sup> Marjan Rahnamaye et al 2009 [21.7%] in spit of that they taking more cases in grade 3<sup>(20)</sup> . Also our study is in agreement with the study by Owens MA et al 2004<sup>(21)</sup> , HER2 amplification ratios by fluorescence in situ hybridization and correlation with immunohistochemistry in a cohort of 6556 breast cancer tissues, In the subset of 6556 tissues analyzed with IHC and FISH, however, 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.

## 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. Finally, 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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