

The Study of HER-2/neu, ER/PR Expression Using Immunohistochemistry (IHC) in the Iraqi Breast Cancer

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Abstract

Background: Breast cancer is the most common cancer in woman that originates from the uncontrolled growth of abnormal breast cells. According to the Iraq Cancer Registry 2009, breast cancer ranks as the first between the ten common cancers in Iraq and there is a wide tendency to increase the rate of breast cancer in earlier age group. Estimation of estrogen (ER) and progesterone (PR) receptors status as well as human epidermal growth factor receptor 2 (Her-2/neu) serve as specific guidance to select the patients whose benefit from endocrine therapy and provide prognostic information. The study aimed to assess the expression of ER/PR hormone receptor and Her-2/neu in breast cancer patients and correlation with various Clinicopathological aspects as a predictive biomarker.

Method: The current study was performed from the period between Jan 2015-Feb 2015 in Department of Oncology that referred to Baghdad Teaching Hospital in the Medical City. Routine staining hematoxylin and eosin (H&E) for histopathological examination and immunohistochemistry (IHC) test for ER, PR and Her-2/neu expression was conducted in all cases.

Results: This study includes total 30 cases of breast cancer. The age of patients were between 29-65 years with 47 ± 9.8 (mean \pm SD). Majority of tumors are invasive ductal carcinomas represented 23(76.7%). There are significant differences between different scores were observed in IHC test for Her-2/neu status and showed 8(26.7%) out of 30 cases were positive expression. The ER and PR expression status was strongly associated and demonstrated in 21(70%) of cases. The most common subgroup was Her-2/neu+, ER/PR+ seen in 10(33.3%) out of 30 breast cancer patients.

Conclusion: HER-2/neu is positively expressed in about 26.7% of breast cancer cases. The study of ER, PR status showed higher rates of positive expression 70% and was strongly associated. Using IHC examination the Her-2/neu+, ER/PR+ subgroup indicates the most common subtype 10 (33.3%) compared to other tumor subtypes in breast cancer.

Keywords: Breast cancer, ER/PR, Her-2/neu, IHC

Background

Breast cancer is the malignant tumor that originates from the uncontrolled growth of abnormal breast cells, mostly from the inner layer of the milk duct or the lobules [1]. It is a wide spectrum cancer in women, include 23% of the female cancers [2], while male breast cancer accounts for less than 1% of all breast cancer diagnoses worldwide [3]. According to the latest Iraq Cancer Registry 2009 [4], breast cancer ranks as the first between the ten common cancers in Iraq and there is a wide tendency to increase the rate of breast cancer in earlier age group.

Breast cancer can be broadly categorized into in situ carcinoma and invasive (infiltrating) carcinoma [5]. The common type of breast cancer, invasive ductal carcinoma, and invades the breast tissue, through progressed form ductal carcinoma in situ. [6].

Although molecular classification is the gold standard for selecting properties of breast cancer and the fundamental technology to expect results [7], but Several histopathological features still have prognostic significance in breast carcinoma such as histologic subtype, Grade, lymph node status, ER/PR status, Growth factor and its receptors [8]. The human epidermal growth factor receptor (HER) family plays a vital role in the development of diseases in human cancers. They control cell growing, continuity, and diversity through many signal transduction pathways and take part in cellular spread and differentiation [9]. The HER-2/neu protein (also known as ErbB-2, c-erbB2 or HER-2/neu) is produced by a proto-oncogene situated on the chromosome 17, translates a 185-kDa trans-membrane tyrosine

kinase receptor protein, which is one of the HER-family (HER1-4) [10,11].

HER-2/neu has shown to have a prognostic value for the treatment with the monoclonal antibody trastuzumab (Herceptin, Roche/Genentech) in breast cancer [11]. Since, earliest study of HER-2/neu as a poor predictive value for breast cancer in 1987 [12], the significance of HER-2/neu oncogene as an adverse prognostic factor has been noted in many other cancers [13,14,15].

HER-2/neu overexpression is a prognostic factor that is associated with aggressive tumor, it mean that HER-2/neu proteins on the surface of cancer cells exhibit highly numbers. Compared to HER-2/neu negative [16, 17]. While in the case of PR/ER status, the ER+ve, PR-ve breast cancers considered a poor prognostic factor compared to ER/PR positive cancers [18]. So, the examine HER-2/neu, ER/PR status expression play acritical role to guide treatment planning.

Methods

Patients and Samples

The current study was performed from the period between Jan 2015-Feb 2015 in Department of Oncology that referred to Baghdad Teaching Hospital in the Medical City. Paraffin-embedded tissue sections of breast carcinoma from 30 female patients who had undergone diagnostic mammography examination for Immunohistochemical (IHC) analysis were included in this study. Agreement of this work was attained from Ethical Committee, Faculty of Medicine.

The clinicopathological features for each patient were obtained from a spread sheet, including age at diagnosis and tumor site other status

were excluded. The histopathological diagnosis was performed by two pathologists at the same hospital.

Immunohistochemical (IHC) analysis

IHC of Her2 protein was achieved on 3 to 4 μm thick paraffin embedded tissue sections placed on poly-L-Lysine coated slides. Deparaffinization, hydration and tissues pretreatment were performed. Staining detection kit /Dako/UK was used to detect protein expression of HER-2/ neu protein according to the manufacturer's instructions. The reagents in this kit have a labeled streptavidin-biotin immunoenzymatic antigen detection system. The immunohistochemistry scores are done according to ASCO/CAP scoring system.

The guidelines of (ASCO-CAP) were utilized to explain of staining and HER-2/neu protein expression was scored as 0 (no stain), 1+ (weak and imperfect membrane staining), 2+ (strong, perfect membrane staining in less than 30% of the invasive tumor cells or weak/moderate heterogeneous complete staining in more than 10% of the invasive tumor cells) , 3+ (strong perfect regular membrane staining in more than 30% of the infiltration tumor cells)[19]. An IHC score of 3+

evidence that a patient's tumor is HER-2/neu positive, while scores of 0 or 1+ elect the tumor is HER-2/ -ve. A score of 2+ is "equivocal," meaning that further testing should be managed using the FISH method [20].

Statistical analysis

Alterations in subjects and tumor features between the numerous breast cancer subtypes and other parameters were evaluated using analysis of System- SAS (2012). Chi-square test was used to significant compare between percentages in this study. (p value < 0.05* was significant; p value < 0.01** was highly significant).

Results

1- Clinicopathological analysis in breast cancer patients

The age of the 30 patients were included in this study ranged between (29-65) years with a mean (SD) of 47 (9.8) and a median of 46 years. Thirteen of the patients (43.4%) within age group (41-50) years was showed significantly high frequency (P = 0.00572) compare to other groups, Table (1). Also the lowest frequency and percentage 3(10%) were clear in age ≥ 65 year.

Table 1: Distribution of age groups in Breast cancer patients

Age Groups	No.	(%)
< 40	7	23.3
41-50	13	43.4
51-60	7	23.3
>60	3	10
Total	30	100
Ch-square	9.217 **	
P -value	0.00572	
** (P<0.01).		

2- Histopathological analysis in breast cancer patients

Cases were predominantly invasive ductal carcinomas represented 23(76.7%) and showed significant differences ($p=0.0038$), with two cases of invasive lobular carcinoma (6.7%) and five cases of ductal mammary carcinoma (16.6%), Table (2).

Table (2): Distribution of cancer histotype in Breast cancer patients

Histotype	No(%)
Invasive ductal carcinoma	23(76.7)
Invasive lobular carcinoma	2(6.7)
Ductal mammary carcinoma	5(16.6)
Total	30(100)
Chi-square	13.269 **
P-value	0.0038

3- Immunohistochemical analysis for HER-2/neu Receptors in breast cancer patients

There are only two possible results for this test: positive, meaning HER-2/neu gene expressed, or negative, indicating the HER-2/ neu gene is not excessive. This study demonstrated that (11/30) 36.6% were score 0 and (3/30) 10% were score+1, which consider as Her-2/neu negative. Out of 30 patients, 8 (26.7%) of them were score+2 which considered equivocal. Also 8/30 (26.7%) were score+3 that reflect as strong positive for Her-2/neu expression. There are significant differences between different scores were observed in this test (Chi-square: 8.257; p value: 0.00749) (Figure 1, 2, A.B.C).

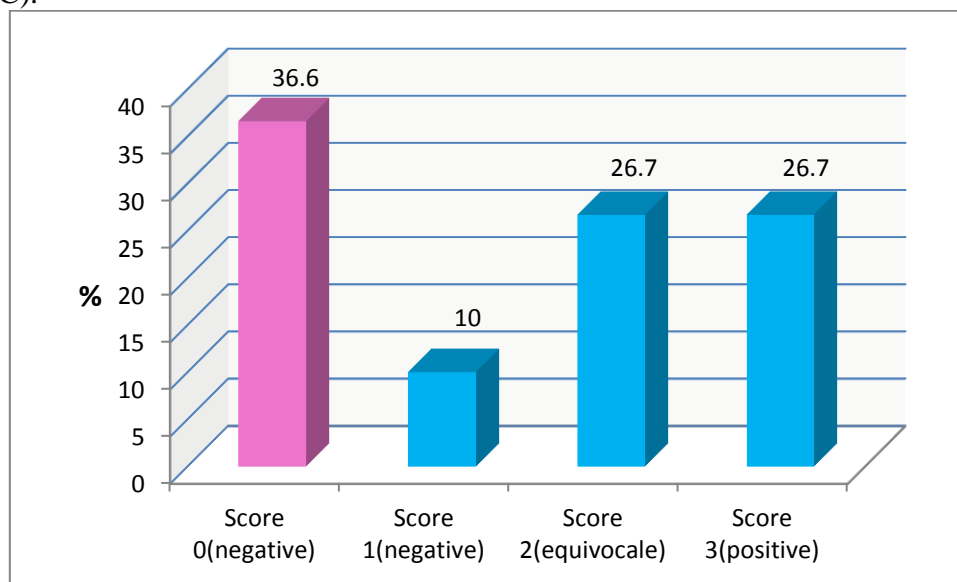


Figure 1: HER-2/ neu percentage score in breast cancer cases using IHC. ($P<0.01$ **))

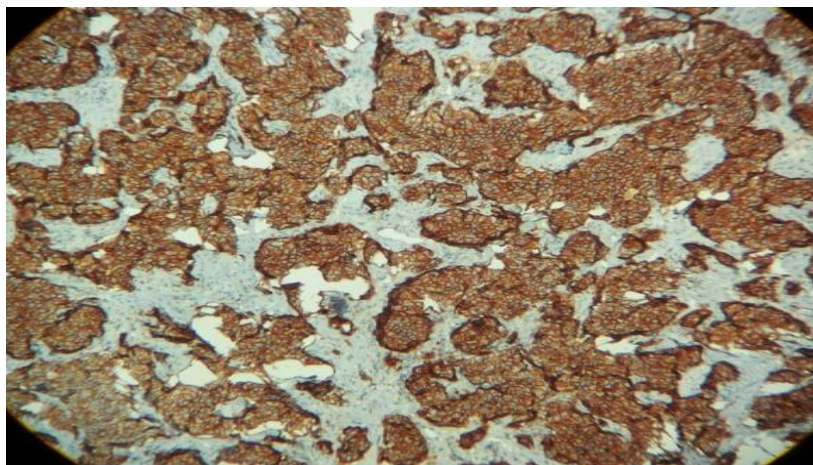


Figure 2: A section of invasive ductal carcinoma stained with anti HER-2/ neu antibody showing pericytoplasmic localization of HER-2/neu Score 3 in malignant cells (40X).

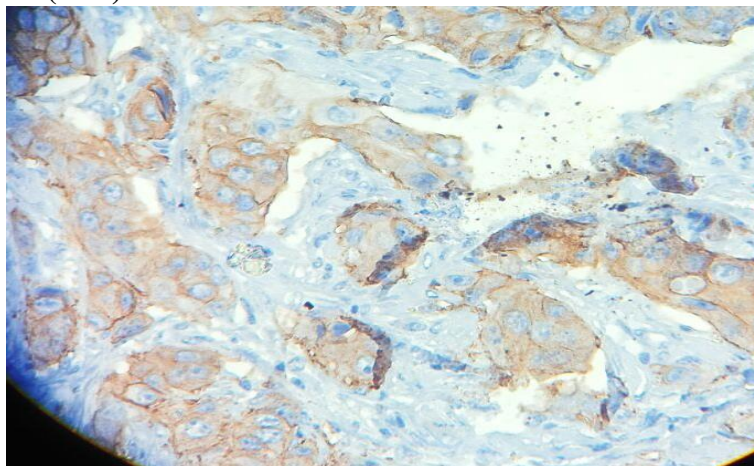


Figure 2B: A section of invasive ductal carcinoma stained with anti HER-2/ neu antibody showing pericytoplasmic localization of HER-2/neu Score 2 in malignant cells (40X).

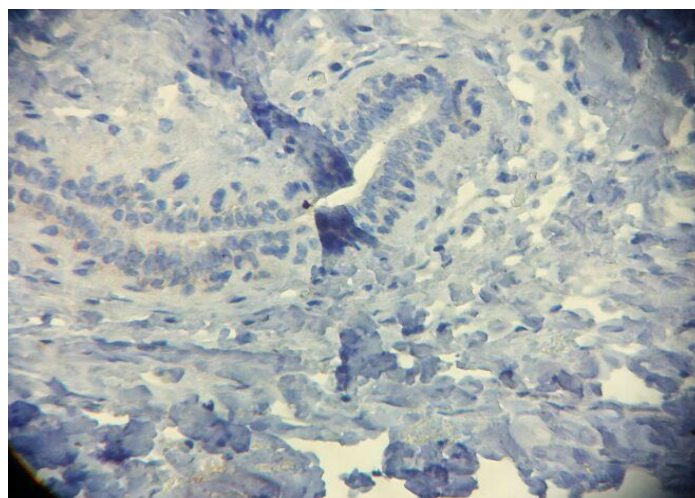


Figure 2C: A cases of breast cancer showing positive immune staining (score 0 Negative) (40X).

4- ER/PR status in breast cancer patients

Among the 30 subjects with ER/PR status, ER expression was strongly associated with PR expression under statistical test ((P value= 0.00174).

Table (3): Distribution of ER/PR statment in Breast cancer patients

ER/PR statment	No.(%)
ER/PR+ve	21(70%)
ER/PR-ve	6(20%)
ER-ve/PR+ve	1(3.3%)
ER+ve/PR-ve	2(6.7%)
P-value	0.00174 **

5- Immunohistochemical subtype classification in breast cancer patients

Standard features of subjects including breast cancer subtype are showing in Table (4). Out of 30 cases, 10(33.3%) were ER/PR+HER-2/neu+ve, 7 (23.3%) were ER/PR+,HER-2/ neu-ve , 4 (13.3%) were ER/PR-,HER-2/neu+ve, and the remaining 2(6.7%) were classified as triple negative Table (4).Subjects with ER/PR+, HER-2/neu+ve subtype were more likely to be significant among other subtype (P-value 0.0117*).

Table (4): Classification of immunohistochemical subtype in Breast cancer patients

Breast cancer subtype	No.(%)
ER/PR, HER-2/ neu+ve	10(33.3%)
ER/PR+ve, HER-2/ neu-ve	7(23.3%)
ER/PR-ve, HER-2/ neu+ve	4(13.3%)
ER/PR-ve, HER-2/ neu-ve	2(6.7%)
p-value	0.0117*

Discussion

HER-2/neu expression are used as a prognostic and predictive biomarker that correlate with poor clinical outcome in breast and other cancers, thus, the treatment directed against HER-2/neu was improved the clinical outcome [15, 21]. In this study, the age group (40-50 years) was reported to have higher frequency in breast cancer patients than others and approximately agreement with many studies [19, 22, 23, 24]. In addition, differed with other who reported the younger breast cancer patients have a higher frequency [25].

Breast cancer is a complex disease comprised different biological subtypes with altered natural history which are progressively documented as giving a diverse range of medical, pathologic and molecular

characterization [26]. The recent study showed the most histological subtype was invasive ductal carcinoma (76.7%), and involved partial quantities of other subtypes, this result supported by Iraqi Cancer Registry 2009 [4]. Also is in agreement with other studies [19, 23, 24]. Breast cancer samples, firstly should undergo HER-2/neu testing by a validated immunohistochemistry (IHC) assay for HER-2/neu protein expression, and HER-2/neu status should be examined in all patients with invasive ductal carcinoma in breast cancer on the basis of 1 or more test results [27]. Our finding showed 8(26.7%) out of 30 subject were HER-2/neu positive and this result is approximately agreement with study that revealed 30% of HER-2/neu is amplified in human breast

cancer [12]. While others showed 42(16%) and 34(13.2%) were HER-2/neu positive, by IHC or FISH, out of 257 and 258 breast cancer patients respectively [19, 20]. Also, HER-2/neu is up regulated in about 15-20% of breast cancer patients by [28, 29]. The positivity of HER-2/neu could be used as a good predictor biomarker that helps to improved clinical outcome. This interpretation was confirmed by study that showed HER-2/neu expression had a reliable prognostic value for axillary lymph node (ALN) metastasis under statistical analysis ($p < 0.0001$) [19]. As well as Amplification of HER-2/neu gene was found to be a significant predictor of both overall survival ($P < 0.001$) and time to relapse ($P < 0.0001$) [15]. Hence, help to consumption treatment with trastuzumab in grouping with chemotherapy [30]. While the HER-2 negative patients provide to be benefit for other treatment than do HER-2/neu positive patients. Consequently, the examined HER-2/neu expression supply very potent information to director treatment development. The HER-2/neu and ER/PR as a three predictive markers have a separated prognostic value, the ER and PR up regulation involved 80-90% and 70-80% respectively of breast-cancer patients [31], while HER-2/neu revealed (13-30%) by [12, 19, 20, 28, 29]. Our data showed that ER expression was powerfully connected with PR expression and demonstrated 21(70%) out of 30 cases of breast cancer. Therefore provide good prognosis for treatment outcome. This result reflected with studies that revealed a strong associated between ER/PR positive expression and showed decrease rate of ALN metastasis when compared to ER/PR negative [19, 24, 32, 33]. While the nonexistence of PR

expression was related with poorer prediction value in ER positive patients treated with other endocrine therapy [33]. Thompson and colleague 2010 [18], showed that the PR-positive is change to PR-negative about (26%) proposing to loss of PR expression during disease progression and hormone therapy unresponsiveness.

Breast cancer subgroup classification using IHC markers, (ER, PR and HER-2-Neu) is widely used in both clinical and researcher field due to its reliability and reproducibility [34], by which can stratify patients into four prognostic groups as we mention above. The preeminent prediction cancers are ER/PR positive and HER-2-neu-negative, while the poorest prediction refer to the triple negative cancers [33, 35]. This stratification of prognosis, already use as accessible bio-markers, in spite of using guideline-based adjuvant therapies [33]. The most subtype in this study was triple positive breast cancer ER/PR+, HER-2/neu+ve 10(33.3%), this subtype practically have a high recurrence score [36]. Other study showed that triple positive and triple negative breast cancer increase risk of ALN metastasis compared to (ER+/PR+, HER-2-ve) subgroups respectively [19].

Conclusion

HER-2/neu is positively expressed in about 26.7% of breast cancer cases. The study of ER, PR status showed higher rates of positive expression 76.7% and was strongly associated. The ER+/PR +Her-2/neu +ve subgroup in breast cancer by IHC technique indicates the most common subtype 10 (33.3%) compared to other tumor subtypes.

Abbreviations

ER: Estrogen Receptor

PR: Progesterone Receptor

Her-2: Human epidermal growth factor receptor -2
 IHC: Immunohistochemistry
 ALN: Axillary Lymph Node

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