



Immunohistochemical Study of Estrogen, Progesterone Receptor and Her-2neu Oncogene with Her-2neu Biomarker Estimation by ELISA Technique in Primary Breast Cancer before Chemical Therapy

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Abstract

Breast cancer is one of the comments malignant tumors worldwide especially in Iraq; it is a leading cause of death in Iraqi women. Determination of estrogen and progesterone receptors status is helpful in selecting the patients most likely to receive benefit from endocrine therapy, and provide prognostic information on recurrence and survival since their expression is related to the degree of the tumor differentiation. From November 2012 to March 2013, 150 breast cancer patients at Al-Amal Hospital in Baghdad were attended to start treatment of disease for the first time. All patients included in this study did not receive chemotherapy. Patients were asked to bring their paraffin embedded tissue blocks to participate in estrogen, progesterone and Her-2/neu receptors estimation. Blood samples were also collected from the patients to estimate positive Her-2/neu cases in serum. Age distribution in women with breast cancer showed that 44% of cases at age group (40 – 49) years. 23% of the patients had a positive family history (first and second degree). Histological types of breast carcinoma showed that 82%, 14% and 4% were ductal, lobular and mixed carcinoma respectively. The Makee classification showed that Grade I, II, and III were detected in 10%, 64% and 26% respectively. TNM staging revealed that 16% of the patients were recorded in stage I, 44% in stage II, 32% in stage III and 8% in stage IV. It was demonstrated that 72% and 70% of breast carcinomas were positive for ER and PR respectively; both markers correlated with age, family history, type, histological grade and stage of the disease. Her-2/neu showed 28% expression in Iraqi breast cancer cases. HER2/neu over-expression (>15 ng/ml) was observed in 36 out of 150 patients (24%) Her-2/neu serum level at ELISA diagnosis and in 42 out of 150 (28%) at immunohistochemistry method (IHC). There were significant (P < 0.001) association between tissue HER-2/neu and serum HER-2 levels. Detection Her-2/neu in serum by ELISA technique could be used as a method for detection with high rate reached to 78% after mastectomy, also it considered as an excellent method for follow up.

Key words: Breast Cancer, ER, PR, Her2/neu

دراسة نسيجية مناعية لمستقبل هرمون الاستروجين والبروجستيرون والهير 2 نيو الورمي مع حساب العلامات البايولوجية للهير 2 نيو عن طريق تقنية الاليزا في مريضات مصابات لسرطان الثدي قبل العلاج الكيميائي

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الخلاصة:

يعد سرطان الثدي احد اكثر انواع السرطان شيوعا في العالم واحد اهم اسباب الوفاة بين نساء العراق ولقد كان لاكتشاف مستقبلات الهرمونات (استروجين و بروجستيرون) فضل كبير في اختيار المريضات للخضوع للعلاج الهرموني ولتوقع فترة العيش بعد العلاج وامكانية رجوع الورم مرة اخرى وذلك للعلاقة بين درجة الورم ونسبة تمثيل مستقبلات الهرمون على سطح الخلايا، يقوم البحث على اساس قياس نسبة ER, PR وHER2neu في النسيج ومصل الدم وملاحظة العلاقة بين مستوى المستقبلات والحالة الاكلينيكية في نساء عراقيات مصابات بسرطان الثدي قبل وبعد الخضوع للعلاج الكيميائي، تم بدأ الدراسة من تشرين الثاني 2012 الى اذار 2013 في مستشفى الامل وتم اختيار المشخصات حديثا بالاصابة بسرطان الثدي وقبل بدأ العلاج الكيميائي للمشاركة في الدراسة ، تم اخذ النسيج المنظمر في البارافين وعينة من الدم من 150 مريضة لمعرفة نسبة الHER2neu الموجبة (اهم بارامتر في الدراسة) 44% من النساء المشمولات في الدراسة كن بين 40-49 سنة، 23% لهن اقارب من الدرجة الاولى والثانية مصابات بالمرض، 82% كان من نوع سرطان القنوات، 14% من نوع قصبي و 4% من النوع المختلط، 64% من الدرجة الثانية، 10% من الدرجة الاولى و 16% من الدرجة الثالثة حسب تصنيف SBR، باستخدام تصنيف TNM 16% في المرحلة الاولى 44% في المرحلة الثانية. 32% في المرحلة الثالثة و 8% في المرحلة الرابعة وكلاهما علامات مرتبطة مع التقدم في العمر والتاريخ العائلي ونوع ودرجة النسيجية ومرحلة المرض. التحليل المناعي immunohistochemistry (over expression) كشف عن 42(28%) حالة زيادة او افراط في التعبير للجين الورمي HER2neu وباستخدام تقنية الفحص المناعي المرتبط بالانزيم ELISA كانت نسبة فرط التعبير 22% بمتوسط يبلغ 17.9 نانوغرام/مل بمدى 12.8_29.9 نانوغرام/مل $r=0.53$, $P \text{ value} < 0.001$. هناك فروقات معنوية ($P < 0.001$) مرتبطة ما بين مستويات HER-2/neu النسيجية و HER-2 مصل الدم. الكشف عن مستقبلات ال Her-2neu في مصل الدم بواسطة تقنية ELISA يمكن أن تستخدم كوسيلة للكشف مع نسبة عالية وصلت إلى 78% بعد عملية استئصال الثدي، كما أنه تعتبرها وسيلة ممتازة لمتابعة العلاج (follow up). تعد تقنية التحليل المناعي اختبار سهل نسبيا ويمكن استخدامه لمراقبة فعالية انسجة الثدي.

Introduction

Breast cancer is the most common cancer in women. Worldwide, more than one million women are affected by this disease every year [1]. In Iraq it constituted 19.59% total cancer cases and alone is accounted for 31% of all new cancer cases among females in Iraq [2].

Several risk factors for the development of the breast cancer have been established, and been proposed that the common denominator for most of these factors is prolonged estrogen stimulation operating on a genetically susceptible background [3]. Numerous studies suggest a strong link between the female hormone, estrogen, and the development of breast cancer, also two to three times increase in the risk of breast carcinoma if a first degree relative has breast cancer [4].

There are risk factors associated with breast cancer development; age, family history, personal history of breast cancer, hormones, cigarette smoking and alcohol consumption [5, 6]. Determination of estrogen and progesterone receptors status is helpful in selecting the patients most likely to receive benefit from endocrine therapy, and provide prognostic information on recurrence and survival since their expression is related to the degree of the tumors differentiation, the highest response rates to endocrine therapy are observed in tumors, which are positive for estrogen and progesterone receptors [7].

The proto-oncogene HER-2/neu is amplified and as a result it is over-expressed in 25% to 30% of human breast cancer and is usually associated with tumor aggressiveness and poor prognosis, Ten In breast cancer, several studies identified the value of analyzing HER-2/neu as an approach to predict the response of individual tumors to chemotherapy as well as in the use of recombinant humanized antibodies (trastuzumab) to the HER-2neu protein in the active management of patients with metastatic breast disease [8].

Immunohistochemistry (IHC) is the most commonly used method of testing for ER, PR, and HER2/neu status [9]. The combined expression of ER, PR and Her-2/neu and some other markers has thus become most informative in the molecular classification of breast tumors and their clinical

assessment for treatment and further outcome [10-12]. HER-2/neu proteins can be found in the body's bloodstream measuring by using available ELISA Kit [12].

Aim of the study:-

Our study can be categorized into three points:

- 1) Prevalence of IHC markers(ER, PR and Her-2/neu) in Iraqi breast cancer women.
- 2) Clinicopathological finding correlated with hormone receptors and oncogene Her-2neu (in both tissue and serum) before and after chemotherapy.

Patients and Methods

Patient:

From November 2012 to March 2013, 150 patients were newly diagnosed as breast cancer and without chemotherapy treatment were included in this study. The patients were diagnosed at Al- Amal Hospital (Hospital Radiation and Nuclear Medicine Previously), Patients were asked to bring their paraffin embedded tissue blocks to participate in the ER, PR and Her-2/neu receptors estimation. Blood samples were also collected from the 150 patients to estimate the positive Her-2/neu cases in serum. Most of these cases had mastectomy with axillary modified clearance mastectomy. The main data and parameter include in our study: patient's age, family history of breast cancer and another cancer, age at first full term pregnancy and pattern of menstrual cycle.

Control:

Ten patients (women) diagnosed as having breast cancer with negative IHC study of Her-2/neu were also included in this study as a control group. Ten apparently normal female individuals with different menstrual status and marital status were also included as a control for serum markers.

Samples Collection:

Blood sampling:

Three to five ml of blood was collected in vacuum tubes from 150 patients and from the ten control individuals. Blood samples were drawn from cubital vein. Blood samples were centrifuged at 3000 rpm for 2-5 minutes then serum was separated and stored immediately in the process of ELISA assay. Specimen held for longer time by frozen at -20°C prior to assay. All samples were obtained after informed consent of the participants prior to their inclusion in the study. A structured questionnaire was used to elicit detailed information on age, age at first full term pregnancy, menstrual cycle and family history of breast cancer and another type of cancer.

Tissue sampling:

Paraffin-embedded tissue blocks of patients were collected. New sections were made from each of the paraffin embedded blocks which included 4-5 μm thickness were made on positively charged slides to be subjected for the purpose of conducting immunohistochemistry procedures to detect ER, PR and Her-2/neu.

Methods:

Immunohistochemistry for ER, PR and Her-2/neu:

Representative paraffin embedded blocks containing breast cancer tissue from all cases were used to obtain sections of 4-5 micron thickness placed on positively charged slides used to assess the estrogen and progesterone receptors and the HER-2/neu status by immunohistochemical staining, together with adjacent breast tissue from the control group (considered as positive tissue control for the ER and PR), in addition to the parallel positive tumor control sections which were processed with each set of staining for the HER-2 immunohistochemistry.

Assay procedure:

- 1- Paraffin-embedded B.M tissue were cut in 4 mm-thick section and placed on super frost charged slides.
- 2- This section is back in hot air at 65°C for overnight or at 80°C for 1 hr.
- 3- the section were deparaffinized in prewarmed xylene (2 x 5 min), rehydration in absolute ,95%,70%,and 30% ethanol for 5 min . Each, then for 5 min .in distilled water.
- 4- the excess water tapped off .any remaining water around the specimen was removed carefully by using soft tissue to keep reagent within prescribed area .Endogenous peroxidase activity was block by covering the section with peroxidase block(3% H_2O_2) for 20-30 min.
- 5- The slides were rinsed gently with distilled water from a wash bottle (have not direct focus flow on tissue) and placed in fresh PBS buffer for 5 min.

- 6- The excess and remaining buffer was removed as in step 4. Enough primary antibodies are applied to cover the section, incubated at 37°C for one hour.
- 7- The slides were rinsed gently with buffer solution from a wash bottle and placed in fresh buffer bath for 5 min. immediately excess buffer is wipe and the slides wiped as before. Enough link antibody (Biotinylated Antibody) was applied to cover the sections, then the slides placed in humid chamber and incubate at 37°C for one hour.
- 8- The slides are rinsed and wipe as in step 4. Enough streptavidin reagents was applied to cover the sections, then the slides place in the humid chambers and incubated at 37 c for 30 min.
- 9- The slides are rinsed and wipe as in step 4. Substrate-chromogen solution (DAB) is freshly prepared by adding one drop of chromogen to one ml. substrate buffer. The section were covered with DAB solution and placed in humid chamber then incubated at 37 c for 10 min.
- 10- The slides are rinsed with distilled water from wash bottle and immersed in bath of myers hematoxyline for 30 sec., the rinsed with running tap water for two min.
- 11- The slides were dehydrated with graded alcohol 30%, 70%, 95%, absolute for 1 min. each, and cleared with xylene 2x for 1min .,then mounted with faramount.

Staining interpretation:

The criteria of positive reaction for ER and PR are dark brown intra-nuclear precipitate. The staining was assessed by scoring the proportion and intensity performed at X 40 objective lens. Allred scoring guideline was used by assessing the proportion score (PS) and intensity score (IS) [13]. As follow:

- 0 (none)
- 1(<1/100)
- 2(1/100-1/10)
- 3(1/10-1/3)
- 4(1/3-2/3)
- 5(>2/3)

Any brown nuclear staining is counted towards the proportion score. An intensity score represented the average intensity of the positive cells, as follows:

- 0 (none)
- 1 (weak)
- 2 (intermediate)
- 3 (strong)

A total score (TS) = sum of PS and IS (0 or 2 – 8). A positive result is defined as TS = or > 3 which was validated in numerous large studies. Score 0-2 considered as negative expression, score 3-4 considered as weak expression, score 5-6 considered as intermediate expression and score 7-8 considered as strong expression.

While Her-2/neu oncogene was different staining of cell membrane in different score as table 1.

Table 1- Scoring for Her-2/neu receptors

Score to report	Her-2 Protein over expression Assessment	Staining Pattern
0	Negative	No reactive or membranous reactivity in < 10% of tumor cell
1+	Negative	Faint/barely perceptible membranous reactivity in >10% of tumor cells; cells are reactive only in part of their membrane
2+	Equivocal	Weak to moderate complete, basolateral membranous reactivity in >10% of tumor cells
3+	Positive	Strong complete, basolateral or lateral membranous reactivity in > 10% of tumor cells

Estimation of serum Her-2ne (Biochemical):

Serum level of Her-2/neu was determined by solid phase enzyme linked immunoassay, using Human ErbB2 (Epidermal Factor Growth Factor Receptor 2) ELISA kit is an *in vitro* enzyme-linked

immunosorbent assay for the quantitative measurement of human ErbB2 in serum (provided by the RayBio_ Company)[14]. Forty two patients (women) diagnosed as having breast cancer with positive Her-2/nue were included in this estimation.

Statistical analysis:

Data were translated into a computerized database structure. The database was examined for errors using range and logical data cleaning methods, an inconsistency were remedied. An expert statistical advice was sought for Statistical analyses were done using minitab 15 computer software statistical software for Six Sigma and quality improvement worldwide. The statistical significance of association between all categorical variables was assessed by Chi-square test of independence. The 95% confidence interval for an estimate (calculated from a sample) gives an idea about the range of values for the corresponding parameter in the reference population with 95% confidence [15].

Results

Clinico-pathological assessment revealed:

The peak age frequency in the total group studied was in the age category of (40-49 years) accounting for 150 patients. In this study 24% of the patients had a positive family history weather it is first or second degree. The histopathology diagnosis showed that a high percentage in Iraqi cases with infiltrated ductal carcinoma which was represented 82%, while the invasive lobular carcinoma was 14% and the mixed carcinoma was 4%. In current study 10% of patients were in grade I, 64% were in grade II and 26% were in grade III; so most of the patients were in grade II and III. The stage of the breast cancer, as in the other types of cancers, is the most important prognostic parameter. In the present study 24 patients (16%) were in stage I, 66 (44%) were diagnosed in stage II, 48 (32%) in stage III. and twelve (8%) in stage IV. Table 2 shows the Clinico-pathological analysis in breast cancer patients.

Table 2- Clinico-pathological analysis in breast cancer patients

Clinico-pathological analysis		
1. Age group/ year (Female)	Number	Percentage
< 29	0	0
30-39	18	12
40-49	66	44
50-59	39	26
≥ 60	27	18
2. Family history	Number	Percentage
Positive patients	36	24
Negative patients	114	76
Total	150	100
3. Type of Breast Cancer	Number	Percentage
Infiltrated Ductal Carcinoma	123	82
Infiltrated Lobular Carcinoma	21	14
Mixed (Ductal& Lobular Ca.)	6	4
Total	150	100
4. Grade	Number	Percentage
Grade I Well differentiation	15	10
Grade II Moderate differentiation	96	64
Grade III Poor differentiation	39	26
Total	150	100
5. Histological feature types of Breast Cancer	Number	Percentage
Stage I	24	16
Stage II	66	44
Stage III	48	32
Stage IV	12	8
Total	150	100

Hormonal status results by IHC

Estrogen and Progesterone Receptor Status in Breast cancer tissues:

ER positive cases were 72 % (108/150) and PR positive cases 70% (105/150). About 50% of positive ER, cases (54 out of 108) had strong positive stain, and 40% of positive PR cases (42 out of 105) had strong positive stain, table 3- and figures 1 & 2.

Her-2 oncogene Status in Iraqi Breast cancer tissues

This study demonstrated that 42 out of 150 malignant cases 28% were positive for Her-2 neu expression. While 8% for Score 1 which consider as Her-2 negative, HER-2 immunohisto scoring 22% (33/150) of patient have strong positive HER-2 that mean HER2 genes are over-producing the HER2 protein and that those cells are growing rapidly and creating the cancer. These breast cancers tend to be much more aggressive and fast growing. While 64% (96/150) had score 0 which mean the HER2 protein is not causing the cancer, table 3- and figure 3.

Table 3- Estrogen, Progesterone Receptors and Her-2neu Status in in Primary Breast Cancer tissues by IHC

Hormonal status		
(ER and PR) Phenotype	Number	Percentage
ER+/PR+	84	56
ER+/PR-	24	16
ER-/PR+	21	14
ER-/PR-	21	14
Total	150	100
Her-2 status		
Her-2 status	Number	Percentage
Score 0 (Negative)	96	64
Score 1 (Negative)	12	8
Score 2 (Positive)	9	6
Score 3 (Positive)	33	22
Total	150	100

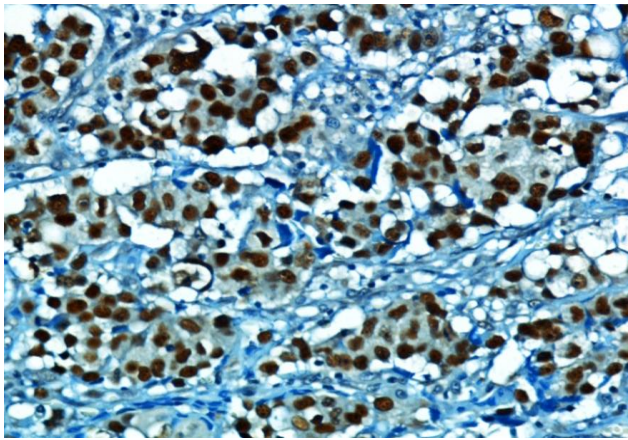


Figure 1- A case of ER in ductal carcinoma showing a strong positive (H&E x40)

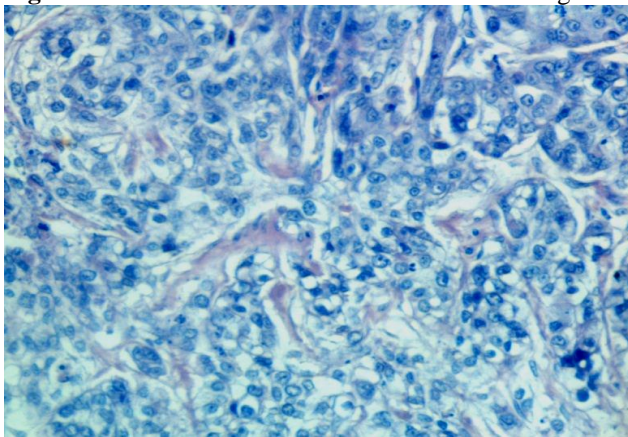


Figure 2- A case of PR in ductal carcinoma showing a strong positive (H&E x40)

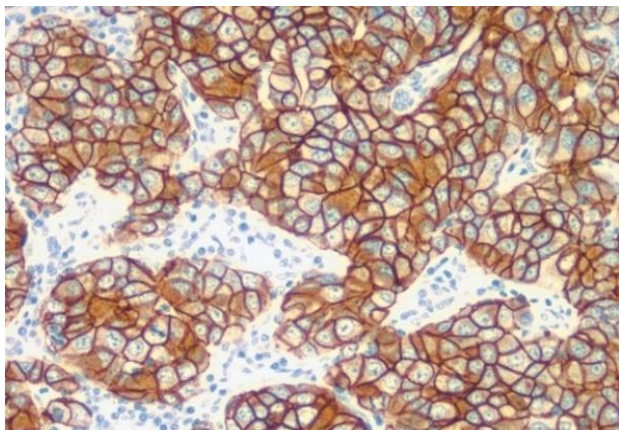


Figure 3-A case of Her-2neu in ductal carcinoma showing a strong positive (score III) (H&E x40)

Correlation between clinico-pathological assessment and IHC tumor markers ER, PR and Her-2/neu

The association between levels of expression of ER, PR, Her-2/neu and age family history, tumor type, tumor grade and stages was summarized in table 3.

For ER, There is no statistically significant association between level of expression of ER and the age, family history, tumor type or stages table 4. The relationships between the levels of expression of PR and the age, family history, tumor type, tumor grade and stages were summarized in Table 4. There is no statistically significant association between levels of expression of PR and the age, family history, tumor type or stages.

The levels of expression Her2/neu didn't show any statistical significant difference with the age, tumor type or tumor grade or the stages positive status except There was a significant correlation between Her-2neu expression and family history ($p < 0.001$), table 4.

Table 4- Correlation between clinico-pathological assessment and IHC tumor markers ER, PR and Her-2neu

Clinico-pathological analysis							
1. Age group/ year (Female)	Number	ER		PR		Her-2neu	
		ER+/+ (100%)	P-value	PR+/+ (100%)	P-value	Her-2neu+ \ (100%)	P-value
50 ≥	89	66(74.15%)	NS 0.636	65(67.7%)	NS 0.807	27(28.1%)	NS 0.973
50 <	61	42(68.85%)		40(74%)		15(27.7%)	
Total	150	108		105		42	
2. Family history	Number	ER+/+ (100%)	P-value	PR+/+ (100%)	P-value	Her-2neu+ \ (100%)	P-value
Positive	36	25(69.4%)	NS 0.115	29(80.5%)	NS 0.327	10(27.7%)	Significant 0.000
Negative	114	83(72.8%)		76(66.6%)		32(28%)	
Total	150	108		105		42	
3. Type of Breast Cancer	Number	ER		PR		Her-2neu	
		ER+/+ (100%)	P-value	PR+/+ (100%)	P-value	Her-2neu+ \ (100%)	P-value
Infiltrated Ductal Carcinoma	123	91(73.9%)	NS 0.836	84(68.2%)	NS 0.946	36(29.2%)	NS 0.820
Infiltrated Lobular Carcinoma	21	14(66.6%)		16(76.1%)		5(23.8%)	
Mixed (Ductal & Lobular Ca.)	6	3(50%)		5(83.3%)		1(16.6%)	
Total	150	108		105		42	
4. Grade	Number	ER		PR		Her-2neu	
		ER+/+ (100%)	P-value	PR+/+ (100%)	P-value	Her-2neu+ \ (100%)	P-value
Grade I Well	15	9(60%)	NS 0.287	8(53.3%)	NS	6(40%)	NS 0.522

differentiation					0.399		
Grade II Moderate differentiation	96	79(82.2%)		76(79.1%)		23(23.9%)	
Grade III Poor differentiation	39	20(51.2%)		21(53.8%)		13(33.3%)	
Total	150	108		105		42	
5.Histological feature types of Breast Cancer	Number	ER		PR		Her-2neu	
		ER+/+ (100%)	P-value	PR+/+ (100%)	P-value	Her-2neu+ \ (100%)	P-value
Stage I	24	16(66.6%)	NS 0.989	20(83.3%)	NS 0.923	4(16.6%)	NS 0.366
Stage II	66	48(72.7%)		46(69.6%)		15(22.7%)	
Stage III	48	36(75%)		31(64.5%)		18(37.5%)	
Stage IV	12	8(66.6%)		8(66.6%)		5(41.6%)	
Total	150	108		105		42	

Serum HER-2/neu results by enzyme linked immuno sorbent assay (ELISA) techniques:

Tissues and serum from 150 patients were assayed by IHC and ELISA and the values are given in Table 5. The age of patients selected for this study ranged from 33 to 80 years. Ten clinically healthy individuals were used as controls.

Immunohistochemical analysis from 150 patient's detected 42 cases with HER-2/neu overexpression (nine cases as Her-2/neu score 2+ and 33 cases as Her-2neu score 3+) and 108 cases were negative for HER-2neu overexpression, figures 4&5.

ELISA method detected 33(78%) cases with HER-2/neu overexpression (≥ 15 ng/ml) from the 42 positive cases (five cases with score 2+ and 28 with score 3+) that already detection by IHC technique, Her-2/neu positive cases which detected by ELISA technique considered a very good rate especially all patient performed after mastectomy and nine cases from score 2+ (equivocal) with median 16.5 ng/ml, range 12.8 to 21.9 ng/ml while Score 3+ Her-2neu positive with median 20.9 ng/ml, range 17.8 to 30.9 ng/ml. A statistically significant correlation ($P < 0.001$, $r = 0.53$,) was observed between serum HER-2neu and tissue HER-2neu.

Table 5- Correlation between HER-2/neu overexpression assayed by IHC and ELISA in 150 patients with breast carcinoma

Her-2/neu status	IHC	ELISA	P Value
Positive II	9(21.4%)	5(11.9%)	Significant $P < 0.001$
Positive III	33(78.6%)	28(66.6%)	
Total	42 (100%)	33 (78%)	

Follow up of serum Her-2/neu after six months of treatment in 10 patients:

To study the level of serum Her-2neu in chemotherapy treated patients we studied follow up cases after 6 months from the commencement of treatment, table 6. It was found that patients showed significantly ($P < 0.05$) reduced serum Her-2neu levels, showing good response to treatment.

There is decrease in level of Her-2neu in serum (below cut off value 15 ng/ml) to all ten cases after six month of post-therapeutic period,

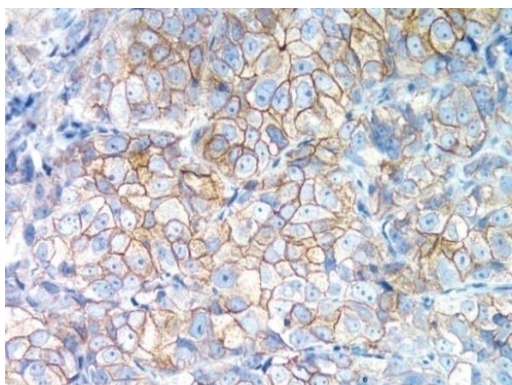


Figure 4- HER-2/neu Positive Case (Score II)

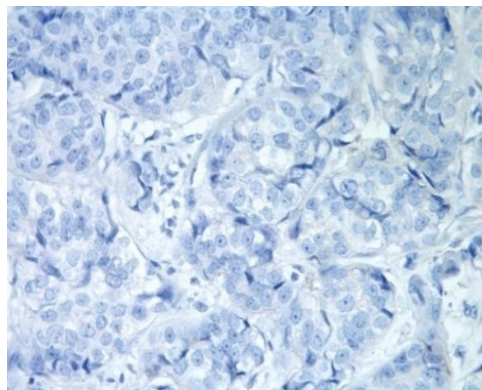


Figure 5- HER-2/neu Negative Case (Score 0)

Table 6- Serum levels of Her-2neu in breast cancer follow-up group after six month (ten patients)

No. of follow up patients	Marker level of Her-2neu			
	> cut off value		< cut off value	
	No. of patients	(%)	No. of patients	(%)
10	0	0	10	100%

Discussion

Breast cancer has a wide range of pathologic aspects and clinical behavior. Breast cancer is either the commonest or second commonest cause of cancer morbidity and mortality among women in developing countries [16]. In Iraq it constituted 19.59% total cancer cases and alone is accounted for (31%) of all new cancer cases among females in Iraq [2]. Numerous studies suggest a strong link between the female hormone, estrogen, and the development of breast cancer, there is two to three times increase the risk of breast carcinoma if a first degree relative has breast cancer [4]. Determination of estrogen and progesterone receptors status is helpful in selecting the patients most likely to receive benefit from endocrine therapy, and provide prognostic information on recurrence and survival since their expression is related to the degree of the tumors differentiation, the highest response rates to endocrine therapy are observed in tumors, which are positive for estrogen and progesterone receptors [7]. The proto-oncogene HER-2/neu is amplified and as a result over expressed in 25% to 30% of human breast cancer and is usually associated with tumor aggressiveness and poor prognosis [17]. HER-2/neu oncoprotein is shed into the serum of normal individuals and is elevated in the serum of many women with metastatic breast cancer [18]. Our studies have utilized a standardized and quantitative manual microtiter plate enzyme linked immunosorbent assay (ELISA) to measure serial serum samples from patients with metastatic breast cancer [19]. HER-2 proteins can be found in the body's bloodstream [20].

ER and PR

Tumors showing positive receptors have better prognosis and better response to hormonal therapy than those with no receptors [21].

In this study demonstrated that 150 malignancy breast carcinoma samples, were include wax blocks embedded tissue. ER positive receptors were in 72 % (108/150) of the cases and PR positive receptors in 70% (105/150) of the cases. Indication there is hormone receptor expression in the majority and cancer is considered hormone-receptor positive and they are likely to respond to hormonal therapies. About 50% of positive ER, cases (54 out of 108) had strong positive stain, and 40% of positive PR cases (42 out of 105) had strong positive stain.

The results of this study were compatible with the Iraqi Cancer Registry 2007 [22]. Findings, they found that Estrogen-receptor positive tumors were noted in 65.1% of the cases and progesterone receptor positive tumors were noted in 45.1% of the cases [23]. In a study on hormone receptor contents of breast carcinoma specimens belong to Iraqi patients reported higher frequencies for ER and PR receptors equivalent to 61% and 52% respectively. On the other hand [24] from Jordanian,

observed in that study that 50.8% and 57.5% of breast cancer samples were positive for ER and PR respectively.

Mammary cancer containing ER+/PR+ phenotype represented the largest category 56% among Iraqi patients. This was followed by ER+/PR- phenotype 16%. ER-/PR- were displayed in (14%) also ER-/PR+ status was found in 14%.

Her2/neu:

In regard to Her2/neu the current results appear to be within the commonly reported rates of 20% to 30% [25-29]. Less than 20% or more than 30% of HER2 over-expression was reported by many studies [30, 31, 32]. This study demonstrated that 42 out of 150 malignant cases 28% were positive for Her-2neu expression. While 8% for Score 1 which consider as Her-2 negative, HER-2 immunohistochemical scoring 22% (33/150) of patient have strong positive HER-2 that mean HER2 genes are over-producing the HER2 protein and that those cells are growing rapidly and creating the cancer. These breast cancers tend to be much more aggressive and fast growing. While 64% (96/150) had score 0 which mean the HER2 protein is not causing the cancer.

Markers and clinicopathological feature

The IHC technique has an expanding prognostic role in determination of factors that affect clinicopathological features. Nevertheless, the results of this study showed different pattern of findings in respect to clinicopathological features. Hormone receptors contents had no noticeable relation with ages, family history, tumor type, tumor grade and histological stages.

ER and PR receptors status have a no statistical significant association with ages, family history, tumor type, tumor grade and histological stages, table 3.

Some authors, as presented in our study, have suggested that HER-2 over-expression is not associated with clinicopathological factors [33, 34]. Furthermore, many authors reported that HER2 hasn't association with histological type [35, 36], tumor grade [37, 38] in contrast, association of HER2 over-expression with tumor grade [26, 32, 39] and histological type [29] was reported. HER-2 has a no statistical significant association with ages, tumor type, tumor grade and histological stages. There was a significant correlation between Her-2neu expression and family history ($p < 0.001$). It is obvious that there is an increasing score with the increase in the family history. In general, 27.7% (10/36).

Correlation between immunohistochemical Analysis and ELISA Her-2neu

The need for accurate detection of the HER-2 alteration has now become even more important, because therapeutic decisions for patients are increasingly dependent on this information [40].

The need for accurate detection of the HER-2 alteration has now become even more important, because therapeutic decisions for patients are increasingly dependent on this information. Moreover, it is currently the sole criteria for the selection of patients for HER-2neu targeted therapy with Herceptin [39].

The "best" method to measure HER-2/neu has become a point of controversy as the value of measuring this marker for making treatment decisions has become apparent [41]. When making individual patient care decisions one test does not always provide all the necessary information. For example, knowing IHC, does ELISA give us any further information, Does the answer depend on whether IHC was positive or negative, In the present study, we analyzed the incidence of HER-2/neu alterations by IHC and ELISA in 150 breast cancer patients Out of the 150 cases that we analyzed 42 cases were found to be overexpressed by IHC. This correlates with the published range of 25-30% of all breast cancer cases [42]. HER-2neu in serum was positive in 22% (33 of 150) of patients.

Advantages of IHC testing include its wide availability, easy preservation of stained slides, and use of a familiar routine microscope [43]. However, variable fixation protocols and subjective grading can cause difficulty in interpretation [44]. The readout for ELISA is relatively rapid compared to IHC. In addition, ELISA eliminates the need for biopsy to avoid the potential antigen damage associated with fixation, embedding, and uncontrolled storage by IHC technique [45]. Also to avoid improperly performed arise by IHC test when antigen is lost during fixation or processing of tissues [41]. There are may be several reasons for this phenomenon. Many laboratories perform the staining on referred specimens and cannot control the time and nature of tissue fixation, the method of tissue processing, or the temperature of the paraffin embedding procedure, all of which can influence HER-2/neu protein antigen loss. Prolonged storage can also be a problem, and significant loss of tumor marker immunostaining intensity has been identified, particularly when specimens are stored as unstained

slides. The impact of the fixative has been considered and shown to have a significant impact on HER-2/neu immunostaining [46]. These problems are significant when IHC is exclusively used to test for HER-2/neu overexpression [47]. In these cases by ELISA might be attributable to transformation of tissue HER-2/neu status at disease recurrence, as has been reported [48].

Furthermore, because there is a correlation between serum HER-2/neu levels and HER-2/neu expression in the tumor, the selection of patients for chemotherapy therapy on the basis of high serum HER-2/neu levels may be considered if tissue is not available [48]. In this study we revealed a statistically significant ($P < 0.001$) association between tissue HER-2/neu and serum HER-2/neu levels. Immunohistochemistry for the detection of HER-2/neu protein overexpression showed a very good correlation ($r = 0.53$) with the results of ELISA. In conclusion our study showed that for routine diagnostics, the combination of IHC and ELISA is useful. Furthermore, ELISA can be used if the primary tissue sample is not available for predicting tissue HER-2/neu status.

After six month of post-therapeutic period, serum Her-2/neu levels in patients who were treated with chemotherapy showed significantly ($P < 0.05$) reduced serum Her-2/neu levels, showing good response to treatment with 100% of the patients had a decrease in Her-2/neu level [49]. Found that three months or more is a good interval for follow up (post therapeutic) and that the value may subside to the pretreated level.

Conclusion:

PR, ER, and Her-2 neu immunohistochemical staining techniques are relatively simple techniques that could be used for measurement of the proliferative activity of the detected breast lesions. A statistically significant correlation was observed. HER-2/neu detected by IHC correlates significantly with serum HER-2/neu levels detected by ELISA. Thus, ELISA is a reliable tool to assess the HER-2/neu status in tumors, when breast tissue sample is not available also in study show low concentration than cut off value after chemotherapy treatment, so it consider as an excellent method for follow up.

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