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**RESEARCH ARTICLE**

# Immunohistochemical evaluation of human epidermal growth factor receptor 2 and estrogen and progesterone receptors in Iraqi breast carcinoma women

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## *Abstract*

The molecular classification for breast carcinomas has been used in clinical studies with a simple surrogate panel of immunohistochemistry (IHC) markers. The objective of this current project was to study the molecular classification of commonly used breast cancer by IHC analysis. Fifty breast cancer samples were collected , fixed in formalin and made into paraffin blocks. IHC analyses were performed on each block with antibodies to estrogen receptor (ER), progesterone receptor (PR) and HER2. Among the 50 samples, Estrogen positive receptors were in 48% (24/50) of the cases, progesterone positive receptors in 46 % (23/50) of the cases and HER2 was 7 of 50 malignant cases (14%) were positive for her-2/neu expression while 74 % for score 0 and score 1 which consider as her-2/neu negative. Luminal A subtype:54% (27/50) ; 4%(2/50) cases that refer to Luminal B subtype ; 10% (5/50) Cases which were referred to HER2 over-expression subtype and 32%(16/50) cases to basal subtype. Since each subtype defined by this IHC-based molecular classification does show a distinct clinical outcome, attention should be paid when choosing this markers for any study.



## INTRODUCTION

Breast cancer is a heterogeneous disease with substantial genotypic and phenotypic diversity(Bågeman et al.,(2008); Luis et al., (2012), It is the most common malignant tumor that occurs in women , it is considered as hereditary disease due to inherited autosomal dominant mutations in several susceptibility genes(Al Ghurabi et al., (2002); Ghanim, M et al.,(2009). Worldwide, about 1.1 million cases are diagnosed annually and the fifth most common cause of cancer death after lung cancer, stomach cancer, liver cancer, and colon cancer in Iraq (Iraqi Cancer Registry Center Publications et al.,2009; Al-Bederi et al.,2011) . Immunohistochemistry refers to the process of localizing proteins in cells of a tissue section utilizing the principle of antibodies binding specifically to antigens in biological tissues (Warford, A et al.,2004). . Immunohistochemistry is widely used in basic research to understand the distribution and localization of biomarkers in the tissues(Kyndi et al.,2008; Al- Bederi et al., 2011).

Tumor markers are substances that can be found in abnormal amounts in the blood, urine and tissues of some patients with cancer(Lemoine et al., 1994). Markers are needed to predict cancer progression and the risk of late recurrence .A small number of single biomarkers, including estrogen receptor (ER), progesterone receptor (PR), human epidermal growth factor receptor-2 (HER2), and proliferation marker Ki-67 have been used for several years (Subik, et al ., 2010) to predict the prognosis of breast cancer and to guide its therapy (Joensuu et al., 2013).

The tumors of approximately 25%–30% of the patients with breast cancer over express HER2 protein (Joensuu et al., 2013), Aberrant expression or activity of two members of the human epidermal growth factor family of receptors (HER), HER1 and HER2, have been connected to 20−30% of breast cancer cases (Yarden.et al ., 2001;Moghimi et al.,2013). HER2 is a signaling tyrosine kinase receptor that causes increased cell proliferation, tumor invasiveness, accelerated angiogenesis and reduced apoptosis, ultimately translating into an aggressive disease, resistant to traditional systemic therapy, increased probability for recurrent disease and decreased survival (Kruser et al., 2010; Emde, et al ., 2011). A crucial development in the treatment of breast carcinoma has been the

realization that the presence of hormone (estrogen and progesterone) receptors in the tumor tissue correlates well with response to hormone therapy and chemotherapy (Hawkins et al.,(1980); Barnes et al ., 2001).

The sex steroid hormone estrogen is important in both men and women for a variety of physiologic processes: growth, differentiation, and function of tissues of the reproductive system. it was shown to be an effective therapy in those patients whose tumors expressed ER (Saez et al .,1980).Progesterone receptor is a sex steroid essential for pregnancy and lactation. Assessment of the role that either progesterone insufficiency or inadequate progesterone response plays in human reproductive failure has been difficult to assess because serum progesterone concentrations fluctuate markedly, limiting the ability to characterize sufficiency of progesterone, and there are no highly reliable markers of endometrial function available (Young et al., 2010).A small number of single biomarkers, including estrogen receptor (ER), progesterone receptor (PR), human epidermal growth factor receptor-2 (HER2), and proliferation marker Ki-67 have been used for several years to predict the prognosis of breast cancer and to guide its therapy (Sørlie et al .,2001). Our aims in this study are to evaluate the expression of ER and PR and the overexpression of Her-2/neu in Iraqi patients with breast cancer, and to compare the expression of these with other prognostic parameters for mammary carcinomas, such as histological type, histological grade, tumor size, patients' age .

## Material and Methods

The samples were collected from AL-SADER Medical city archive and some private laboratory. Fifty invasive ductal carcinomas were used of formalin-fixed paraffin embedded breast cancer from females aged 21-75 years. Four µm sections of multi-block were taken by ELICA Microtome fixed in slides. The immunosatining method used in the current study was Labeled Strept-Avidin Biotin ($LSAB^+$) technique which was applied for HER2, ER and PR staining according to manufacture protocol of DAKO company (Climent et al .,2001). Sections were cut at 4 µm thicknesses, mounted onto silanized slides, and left to dry overnight at 37°C. Sections were then deparaffinized and rehydrated. Antigen retrieval was achieved by heat retrieval using a bench autoclave. Briefly, slides were placed in Coplin jars containing enough 0.01 M sodium citrate solution (pH 6.0) to cover the sections, then autoclaved at 121°C for 10 minutes in the case of Her-2, and 15 minutes for both ER and PR. Slides were incubated with 100–200µl of primary antibodies for 30 minutes at room temperature in a moisture chamber, then rinsed in PBS. The dilution of the primary antibodies against ER and PR (Biogenex,San Ramon, Ca, USA) was 1:130, and for Her-2/neu (Dako, Carpintera, Ca, USA) 1:50. After washing, binding of antibodies was detected by incubation for 10 minutes with biotinylated goat anti-mouse antibody ready to use (LSAB2) from Dako; the slides were then rinsed with PBS. Sections were then incubated with streptavidin-horse radish peroxidase for 10 minutes. Finally, the sections were washed in 4 times in 4minute changes of PBS, followed by adding 3,3 diaminobenzidine tetra hydrochloride (Biogenic) as a chromogen to produce the characteristic brown stain.For each run of staining, a positive and negative control slide were also prepared. The positive control slides were prepared from breast carcinoma known to be positive for the antigen under study. The negative control slides were prepared from the same tissue block, but incubated with PBS instead of the primary antibody.

A semi-quantitative histochemical score was used to record results of ER and PR staining according to the system established by Allred et al [1998]. This system considers both the proportion and intensity of stained cells. The intensity score (IS) ranges from 0 to 3, with 0 being no staining, 1 weak staining,2 intermediate staining, and 3 intense staining. The proportion score (PS) estimates the proportion of positive tumor cells and ranges from 0 to 5, with 0 being non-reacting, 1 for 1% reacting tumor cells, 2 for 10% reacting tumor cells, 3 for one-third reacting tumor cells, 4 for two-thirds reacting tumor cells, and 5 if 100% of tumor cells show reactivity. The PS and IS are added to obtain a total score (TS) that ranges from 0 to 8.Tumor cells with a total score of 3 to 8 were considered positive, whereas those with a TS less than 3 were considered negative cases.

Her-2/neu was scored on a 0 to 3 scale according to the criteria set by Dako. The staining was scored as: negative (0) when no membrane staining was observed, or when membranous staining was observed in less than 10% of the tumor cells; weak positive (1+) if weak focal membrane staining was seen in more than 10% of the tumor cells; intermediate (2+) if weak to moderate, complete membrane staining was seen in more than 10% of the tumor cells; and strongly positive (3+) if intense membrane staining with weak to moderate cytoplasmic reactivity was seen in more than 10% of the tumor cells. Figure 1 illustrates scores 1+, 2+, and 3+ as uses in this study. In the final analysis, however, scores 0 and 1 were considered negative; score 2 was considered weakly positive; and score 3 was considered strongly positive. Only score 3 cases were considered as Her-2 over expressing cases.

The Student's t-test was used for comparison of mean tumor size and mean patient age for each category of cases. The chi square test was used to compare the expression of ER, PR and Her-2 among different cases, including: those above or below 50 years of age; those with tumor size up to 2 cm with those between 2 and 5 cm and those larger than 5 cm in size. The results were considered statistically significant if the P value was $< 0.05$.

## Results and Discussion

The peak age frequency in the total group studied was in the age category of (41-50 yr) accounting of 50 patients. In this study more than 24% of the patients have family history either it is the first or second degree. The histopathology diagnosis showed that a high percentage in Iraqi cases with infiltrated ductal carcinoma represented (86%),while the invasive lobular carcinoma represented(4%) and the mixed carcinoma represented (10%) which that agreement approximately well with Groheux (Groheux et al .,2013). In current study 12 % of patients were in grade 1, 20 % were in grade 2 and 68 % were in grade 3 , so most of our patients were in grade 2 and 3. The stage of the breast cancer as in the other types of cancer is the most important prognostic parameter.

The present results on Iraqi women patients revealed that a high age frequency of cancer occurred between 41-50 years of age (46%), that corresponding with other demographic Iraqi studies revealed that the age range accounting for 67 out of 216 breast cancer patients (31%) was most frequent in Iraq and in agreement with the results of the present study, a group of researchers both in USA and Australia have found that the incidence of breast cancer increasing sharply after the age of 40 (Wu et al .,2002; Edwards et al.,2002) ,while some studies differ in their results which show high age frequency between 51-60(38.89%) as reported by Jumaah et al., (2013). The results of this study could explain the effect of hormonal status in female patient that play an important role in the behavior of the disease.

The results observed that all samples from patients under study included embedded formalin fixed blocks tissue. Estrogen positive receptors were in 48% (24/50) of the cases and progesterone positive receptors in 46 % (23/50) of the cases, and these data revealed that presence of hormone receptor expression in the majority and cancer was considered hormone receptor positive and they were likely responded to hormonal therapy. These results exactly agree with that mentioned by Joensuu et al.,(2013). About 56 % of positive ER cases had strong positive stain and 42 % of positive PR cases had strong positive stain as observed in table 2 and figures 1,2 and 3.

**Table ( 1 ) :** Total number of malignant breast cancer cases with their Hormonal Levels

| No. of samples | Age Years/old | HER2 | I | P | T | ER | I | P | T | PR |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 52 | Negative1+ | | | | negative | | | | negative |
| 2 | 46 | Negative | 2+ | +3 | 5 | Positive | 3+ | 4+ | 7 | Positive |
| 3 | 47 | Positive 3+ | 3 | 1 | 4 | Positive | 3 | 3 | 6 | Positive |
| 4 | 50 | Negative | 3 | 2 | 5 | Positive | 3 | 4 | 7 | Positive |
| 5 | 40 | Negative | | | | Negative | | | | Negative |
| 6 | 52 | Negative | | | | Negative | | | | Negative |
| 7 | 51 | Negative | | | | Negative | 1+ | 2+ | 3 | Positive |
| 8 | 48 | Negative | | | | Negative | | | | Negative |
| 9 | 43 | Negative | | | | Negative | 4 | 2 | 6 | Positive |
| 10 | 31 | Positive + 3 | | | | Negative | | | | Negative |
| 11 | 50 | Negative | 1 | 2 | 3 | Positive | | | | Negative |
| 12 | 62 | Negative | | | | Negative | | | | Negative |
| 13 | 50 | Negative1+ | | | | Negative | | | | Negative |
| 14 | 75 | Negative | | | | Negative | | | | Negative |
| 15 | 32 | Positive 3+ | | | | Negative | | | | Negative |
| 16 | 50 | Negative | | | | Negative | 1 | 2 | 3 | Positive |
| 17 | 45 | Negative | | | | Negative | | | | Negative |
| 18 | 52 | Equivocal2+ | 3 | 4 | 7 | Positive | | | | Negative |
| 19 | 44 | Negative | 3 | 4 | 7 | Positive | 3 | 3 | 6 | Positive |
| 20 | 70 | Negative | 2 | 2 | 4 | Positive | 2 | 4 | 6 | Positive |
| 21 | 50 | Negative | 3 | 3 | 6 | Positive | 2 | 3 | 5 | Positive |
| 22 | 80 | Negative 1+ | 3 | 1 | 4 | Positive | 2 | 1 | 3 | Positive |
| 23 | 63 | Equivocal2+ | 3 | 4 | 7 | Positive | 2 | 2 | 4 | Positive |
| 24 | 42 | Negative | | | | Negative | | | | Negative |
| 25 | 40 | Negative | | | | Negative | | | | Negative |
| 26 | 70 | Negative | 2 | 2 | 4 | Positive | 2 | 3 | 5 | Positive |
| 27 | 21 | Equivocal2+ | 1 | 3 | 4 | Positive | 2 | 3 | 5 | Positive |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| 28 | 50 | Positive 3+ | | | | Negative | | | | Negative |
| 29 | 62 | Negative | 2 | 3 | 5 | Positive | 1 | 2 | 3 | Positive |
| 30 | 60 | Equivocal2+ | 1 | 3 | 4 | Positive | | | | Negative |
| 31 | 46 | Negative | 2 | 2 | 4 | Positive | 1 | 3 | 4 | Positive |
| 32 | 50 | Negative | 1 | 3 | 4 | Positive | | | | Negative |
| 33 | 25 | Positive+3 | | | | Negative | | | | Negative |
| 34 | 58 | Negative | | | | Negative | | | | Negative |
| 35 | 42 | Negative | | | | Negative | | | | Negative |
| 36 | 43 | Negative | | | | Negative | 2 | 1 | 3 | Positive |
| 37 | 67 | Negative | 3 | 1 | 4 | Positive | 1 | 3 | 4 | Positive |
| 38 | 34 | Negative | | | | Negative | | | | Negative |
| 39 | 60 | Equivocal2+ | 3 | 3 | 6 | Positive | 2 | 2 | 4 | Positive |
| 40 | 65 | Negative | 1 | 2 | 3 | Positive | 2 | 3 | 5 | Positive |
| 41 | 50 | Negative | | | | Negative | | | | Negative |
| 42 | 42 | Equivocal2+ | 2 | 3 | 5 | Positive | | | | Negative |
| 43 | 62 | Negative | 1 | 2 | 3 | Positive | 2 | 2 | 4 | Positive |
| 44 | 48 | Positive+3 | | | | Negative | 3 | 4 | 7 | Positive |
| 45 | 76 | Negative | | | | Negative | | | | Negative |
| 46 | 36 | Negative | | | | Negative | | | | Negative |
| 47 | 45 | Negative | 1 | 3 | 4 | Positive | | | | Negative |
| 48 | 70 | Negative | 2 | 3 | 5 | Positive | 2 | 2 | 4 | Positive |
| 49 | 50 | Positive+3 | | | | Negative | | | | Negative |
| 50 | 52 | Negative | 1 | 3 | 4 | Positive | 2 | 3 | 5 | Positive |

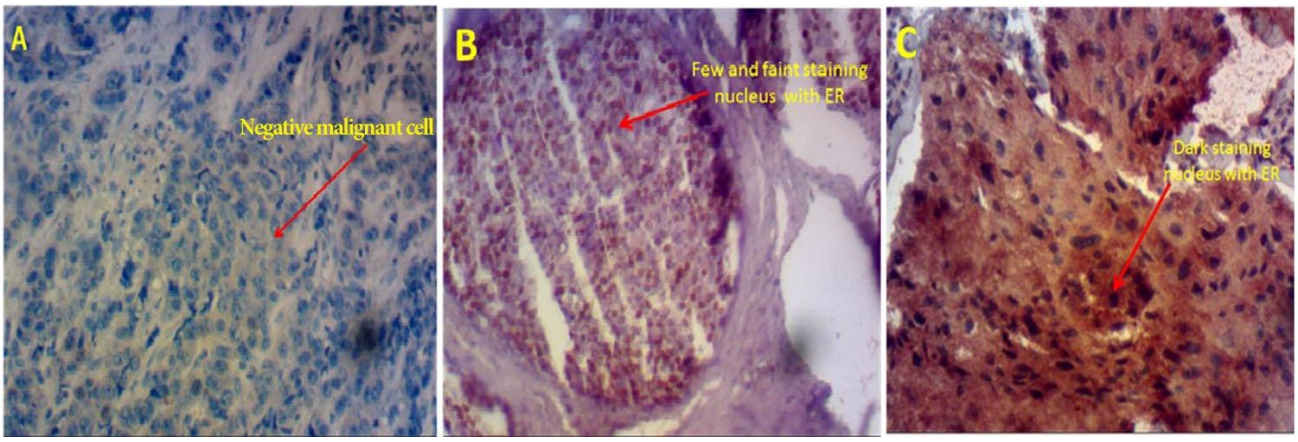


**Figure (1): Representative staining results from H&E and IHC: ( A) : Negative Staining for ER in malignant breast cell magnification 10X (score 0), (B) : ER staining in a metastatic breast cancer , magnification 10X ( score 2), (C): ER staining in a metastatic breast cancer , magnification 40 X ( score 3).**

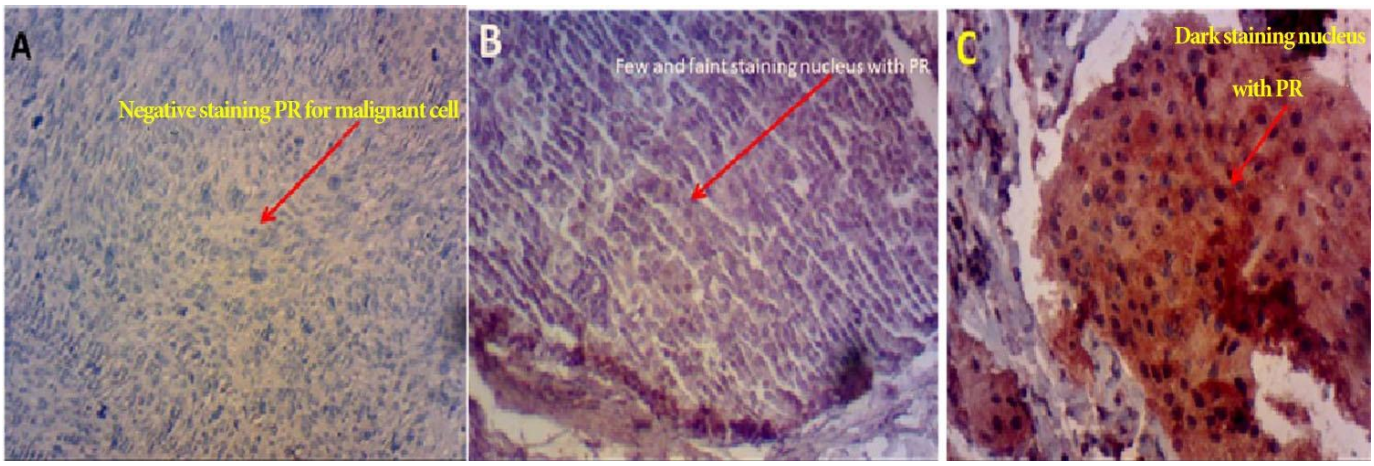


**Figure ( 2 ): Representative staining results from H&E and IHC: (A) : Negative Staining for Progesterone receptor in malignant breast cell, ( B) : PR Positive staining in a metastatic breast cancer, magnification 10X, (C): PR Positive staining in a metastatic breast cancer , magnification 40 X .**

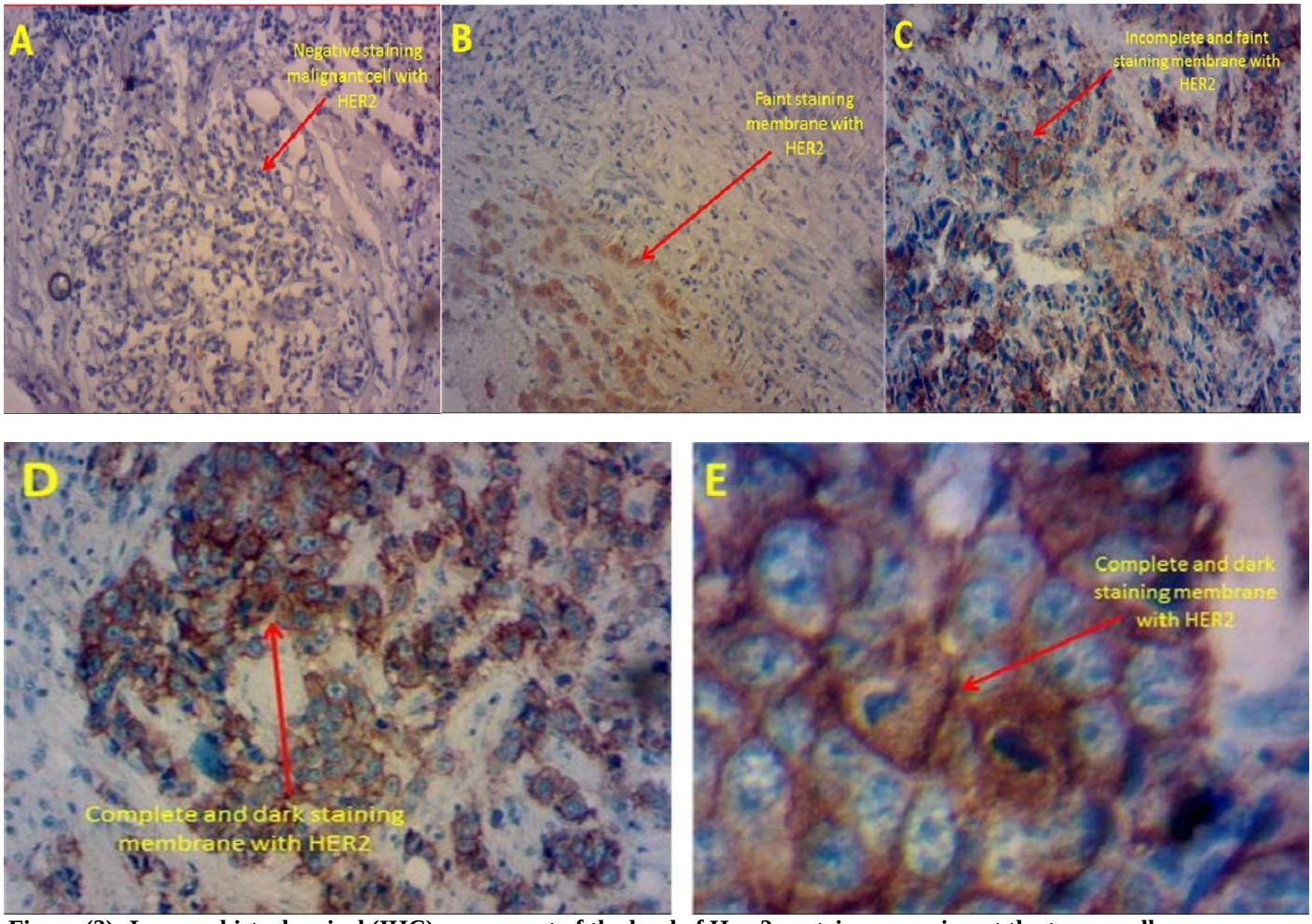


**Figure (3): Immunohistochemical (IHC) assessment of the level of Her-2 protein expression at the tumor cell membrane using the approved DAKO Hercep Test kit according to the manufacturer's instructions.(A) Cases with no membrane staining are scored as IHC 0.**

The association between the levels of expression of ER,PR and Her-2/neu and the age ,family history, tumor grade ,tumor type and stage was summarized in table (3-1). For ER there is no statistically significant association between levels of expression of ER and the age, family history, tumor types or stages (P>0.05). The relationships between the PR levels of expression and the age, family history, tumor grade, tumor type and stage was also not significant (P>0.05).On the other hand the expression of Her-2/neu didn't show any statistical significant difference with the age, tumor grade or type except there was a significant correlation between her2/neu expression and family history (P<0.01).

Tumor showing positive receptors has better prognosis and better response to hormonal therapy than those with no receptors (Rosia et al., 2004). This study demonstrated 50 malignancy breast carcinoma samples were included wax blocks embedded tissue. ER receptors were in % 48(24/50) of the cases and PR positive receptors in (46)% (23/50) of the cases so we concluded that there was hormone receptor expression in the majority of breast cancer in Iraqi patients under study and breast cancer was considered hormone receptor positive and they were likely respond to hormonal therapies. About 56 % of positive ER had a strong positive stain while 42% of PR had strong positive stain.

The results were compatible with the Iraqi cancer therapy registry (Iraqi center Board 2007) findings. They found that ER positive tumors were noted in 65% of the cases and PR positive tumors were noted in 45% of the cases. In a study on hormone receptor contents of breast carcinoma specimens belong to Iraqi patients reported higher frequencies for ER and PR equivalent to 61% and 52% respectively(Al-Alwan et al., 2000). One the other hand (Sughayer et al.,2006) from Jordanian study found that 50% and 57% of breast cancer samples were positive for ER and PR respectively.

According to the classification of molecular subtype of breast cancer , the results show that a group with ER+, PR+ and HER2- were 34%(17/50) cases, also when ER or PR is negative and HER2-) is ( 10/50 ) which represented Luminal A group 54%(27/50), then a group with triple negative ( ER-, PR- and HER2- ) is 32%(16/50) cases that represent Basal like group , while the other groups appeared least frequency as following :( HER +,ER- and PR- ) = 5 Cases which were referred to HER2 subtype group ,then ( HER+ , ER+ and PR+)= 1 Case only and (HER+ , ER or PR + ) = 1 case also that refer to Luminal B subtype. So the classification of the subtypes of breast cancer for this study observed in table 2 .:

In regard to her2/neu the current results appear to be lower than that completely reported rates of 20 % to 30 % (Al-masri et al., 2005; Rashed et al., 2007 ; Azizun-Nisa et al., 2008; Lu et al., 2008 and Mudduwa ,2009), while it compatible well with that reported by other workers (Yarney et al.,2008; Adebamowo et al., 2008 and Cho et al., 2008). The present study demonstrated that 7 out of 50 malignant cases 14 % were positive for her2/neu expression, while 37 cases out of 50 were with score 0 and score1 and 6 cases with score 2 considered (equivocal ) as her2 negative.

The study was focused on The relationship between hormonal receptors and Her-2/neu status and subsequently correlated the results with the studied clinical and morphoclinical parameters. The study determined that 28.5%(2/7) of cases with positive Her-2/neu of score 3+ were also positive for both hormonal receptors. On the contrary, 71.4% of the cases with positive Her-2/neu were characterized by the absence of nuclear stain for both estrogen and progesterone receptors. Analysis of the relationship between the response to hormonal receptors and Her-2/neu status allowed the distribution of 50 breast cancer cases into molecular classification, using "surrogate Immunohistochemical criteria".6 cases (12%) with equivocal Immunohistochemical stain (Her-2/neu score 2+) were excluded. Thus, we obtained the following incidences of molecular subtypes: luminal A subtype, with the greatest incidence 54% of cases, followed by basal subtype with 32%. Her-2/neu subtype represented 10% of cases and Luminal B had the lowest incidence, respectively 4% of cases (table3-6).

The analysis of the obtained correlations between molecular subtypes and morphoclinical parameters was done and the results observed that luminal A subtype characterized the groups aged over 50 (p<0.05). The majority of cases belonging to luminal B were aged under 50 (p<0.05). Regarding Her-2/neu cases, 40% were found in patients aged under 50 and 60% at the group aged 51-60. There was no case over the age of 60.The basal group was met in 90% of the cases aged over 50(p<0.05).This study investigated the relationship between molecular subtypes and tumor size and we observed that tumors of small sizes (T1-T2) prevailed (92% of the cases) in luminal A. Regarding luminal B subtype, 83.4% of cases had dimensions classified in T2-T3. Tumors in the subtypes with negative hormonal receptors had large sizes specific to T2-T3 (60% of Her-2/neu tumors, respectively 91% of basal subtype. As concerns tumor differentiation degree, luminal A subtype was associated in percent of 58% of cases with well and moderately differentiated tumors. Luminal B subtype presented low differentiated tumors in 66.7% of the cases. Her-2/neu subtype was associated in 80% of cases with low-differentiated tumors G3. Basal subtype presented a moderate and low differentiation degree in 91% of cases. The correct treatment of breast cancer is a

multidisciplinary treatment, the sequence of therapeutic methods and their aggressiveness being conditioned by the histopathological type, tumor sizes, adenopathies, the patients` age and their menopause status. The specific markers ER, PR and Her2/neu are used in treatment response prognosis and in guiding the therapeutic plan.

The majority of patients in the study group were aged over 40 (46%). Most tumors had large sizes, 48.9% of the cases were categorized in T2 and T3. 68% of cases were low-differentiated, classified in G3 category, comparatively with only 12% of cases of well differentiated tumors G1. The histological grade and the nuclear grade are prognostic factors useful for the stratification by stage, especially for cases without lymph node metastases **(**Rosen et al., 1981). Evaluation of hormonal status determined that 48% of cases in this study groups were ER+ and 52% ER-, respectively 46% were PR+ and 54% PR-,results which can be compared with those obtained by large studied groups and published in the specialty literature (Rakha et al., 2007) . aged 51-60. There was no case over the age of 60.The basal group was met in 90% of the cases aged over 50($p<0.05$).This study investigated the relationship between molecular subtypes and tumor size and we observed that tumors of small sizes (T1-T2) prevailed (92% of the cases) in luminal A. Regarding luminal B subtype, 83.4% of cases had dimensions classified in T2-T3. Tumors in the subtypes with negative hormonal receptors had large sizes specific to T2-T3 (60% of Her-2/neu tumors, respectively 91% of basal subtype. As concerns tumor differentiation degree, luminal A subtype was associated in percent of 58% of cases with well and moderately differentiated tumors. Luminal B subtype presented low differentiated tumors in 66.7% of the cases. Her-2/neu subtype was associated in 80% of cases with low-differentiated tumors G3. Basal subtype presented a moderate and low differentiation degree in 91% of cases. The correct treatment of breast cancer is a multidisciplinary treatment, the sequence of therapeutic methods and their aggressiveness being conditioned by the histopathological type, tumor sizes, adenopathies, the patients` age and their menopause status. The specific markers ER, PR and Her2/neu are used in treatment response prognosis and in guiding the therapeutic plan

The clinical activity of anti-HER2 agents has been limited to patients with HER2 tumors as defined by intense membrane staining with HER2 antibodies in the majority of tumor cells (3+ by IHC) or 2 copies of the HER2 gene determined by FISH. In general, HER2 IHC and FISH correlate with each other to IHC to reproducibly assess tumors for HER2 overexpression at outside and/or local laboratories for entry into clinical trials . This study demonstrated that 7 out of 50 malignant cases (14%) were positive for her-2/neu expression while 74 % for score 0 and score 1 which consider as her-2/neu negative (figure 3A,B ), and 12% for score 2 which considered equivocal (figure 3C), Her-2/neu Immunohistochemical scoring +3 were 14 % of patients have strong positive ( figure 3 D,E) which mean that her-2 genes are over producing the her-2 protein and that those cell growing rapidly and causing cancer. These breast cancer cases tend to be much more aggressive and fast growing while 60% (33/50) had score 0 which mean that her-2/neu protein is normal producing and not causing cancer. That's where these results near consistent with that is mentioned by Penault-Llorca and Viale, (2012 )When they review estimated that the rate of this discordance is around 7%–26% for HER2 status as these shown in table (2)**.**

**Table ( 2 ):The results of IHC analysis for all breast cancer subtypes according to molecular subtype classification**

| subtype | ER and/or PR | HER2 overexpression | No. of Cases | % |
|---|---|---|---|---|
| Luminal A | + | - | 27 | **54** |
| Luminal B | + | + | 2 | **4** |
| HER2 subtype | - | + | 5 | **10** |
| Basal like | - | - | 16 | **32** |
| Total | | | 50 | **100** |

In this study, we found that 5 (10%) of 50 cases were Her-2 positive. Although there is a wide variation in Her-2 overexpression and amplification, our figure appears to be low within the commonly accepted rate of 20% to 30% (Slamon et al., 1989; Pauletti et al .,1996; Pauletti et al.,2000; Varshney et al., 2004). It does appear, however, to be lower than those reported in East Asia (Yang et al,1997 ;Choi et al .,2004)and in neighboring countries such as Lebanon (Abadjian et al .,1996) and Egypt (Khalifa et al., 2000). Her-2 was expressed in 28% of the infiltrating ductal carcinoma cases compared to only 14% of our seven obular carcinoma cases. This pattern of low Her-2 expression in lobular carcinoma is in agreement with data reported in the literature (Hoff et al 2002 ; Arpino et al .,2004). None of the other types of breast carcinoma showed evidence of Her-2 expression.

We found a clear negative correlation between Her-2 overexpression and age in this study. The mean age of Her-2 positive patients was 11 years less than those patients lacking Her-2 expression, a statistically significant difference. Similarly, patients younger than 50 years of age were 2.6 times more likely to overexpressed Her-2 than patients 50 years of age or older (34% versus 13%). It should be pointed out that higher rates of Her-2 overexpression in young patients have been documented in previous studies(Eppenberger-Castori et al .,2002;Taucher et al.,2003). Our results show a tendency of Her-2 overexpression to be more associated with larger tumor size. Tumors expressing Her-2 were on the average 0.7 cm larger than those lacking Her-2 expression, although this difference was not statistically significant. Similarly, the fraction of tumors larger than 5 cm tended to have higher rates of Her-2 expression than those 2 to 5 cm in size (35% versus 22%), but this difference was not statistically significant (P = 0.13).

## Conclusion

We have shown for the first time that Her-2 is expressed in approximately 10% of breast carcinomas in Iraq. This expression is strongly associated with some known bad pathological and clinical prognostic factors, such as young age, large tumor size and lack of ER and PR expression. In contrast, Estrogen positive receptors were in 48% of the cases and progesterone positive receptors in 46 % of the cases, and these data revealed that presence of hormone receptor expression is the majority and cancer was considered hormone receptor positive and they were likely responded to hormonal therapy

## References

**Al- Bederi, F.M.** (2011). Immunohistochemical Study of Breast Carcinoma in early age patients as compared with middle age by application of BRCA1 and P53 in AL-NAJAF**.** MS.c. thesis **.**Kufa University..pp.126.

**Abadjian** G, Antoun R: Breast carcinoma: evaluation of hormone receptors and pS2, erb-B2, P-glycoprotein and Ki-67 markers. J Med Liban 1996, 44:10-15.

**Al Ghurabi**, S. (2002). DU pollution in southern Iraq after ten years, Proceedings of the Conference on the effects of the use of DU weaponry on human and environment in Iraq, published in Arabic, Vol. 1, March 26-27, Baghdad,

**Allred** DC, Harvey JM, Berardo M, Clark GM: Prognostic and predictive analysis. Mod Pathol 1998, 11:155-168.

**Alwan**, N.A.S. (2010). Breast cancer: demographic characteristics and clinico-pathological presentation of patients in Iraq. Eastern Mediterranean health journal; 16(11): 1159–1164.

**Arpino** G, Bardou VJ, Clark GM, Elledge RM: Infiltrating lobularcarcinoma of the breast: tumor characteristics and clinical outcome. Breast Cancer Res 2004, 6:R149-156.

**Bågeman, E. (**2008). Genetic polymorphisms in breast cancer in relation to risk and prognosis. thesis ph.D. Lund University, Lund, Sweden . pp.85.

**Barnes, DM**. and Hanby, AM. (2001). Estrogen and progesterone receptors in breast cancer: past, present and future .Journal of Histopathology . 38:271-274.

**Climent,** M.A.; Seguí, M. A. ; Peiró, G.; Molina, R.; Lerma, E.; Ojeda, B. (2001). Prognostic value of HER-2/neu and p53 expression in node-positive breast cancer. HER-2/neu effect on adjuvant tamoxifen treatment. The Breast . 10 ( 1 ) . 67-77.

**Choi** DH, Shin DB, Lee MH, Lee DW, Dhandapani D, Carter D,King BL, Haffty BG: A comparison of five Immunohistochemical biomarkers and HER-2/neu gene amplification by fluorescence in situ hybridization in white and Korean patients with early-onset breast carcinoma. Cancer 2003, 98:1587-1595.

**Edwards,** B. K. ; Howe, H. L. ; Ries, L. A. ; Thun, M. J. ; Rosenberg, H. M. ; Yancik, R. ; Wingo, P. A. ; Jemal, A. and Feigal, E. G. (2002). Annual report to the nation on the status of cancer, 1973-1999, featuring implications of age and aging on U.S. cancer burden. Cancer .Vol . 94 : 2766-2792.

**Eppenberger-Castori** S, Moore DH Jr, Thor AD, Edgerton SM,Kueng W, Eppenberger U, Benz CC: Age-associated biomarker profiles of human breast cancer. Int J Biochem Cell Bio 2002,34:1318-1330.

**Ghanim, M**. (2009). A Molecular Study of Loss of Heterozygosity in Tissue Samples Isolated from Breast Cancer Patients in Relation with Their Sex Hormones Status. M.Sc. thesis. Baghdad University.pp.102.

**Groheux, D.;** Giacchetti, S. ; Hatt, M. ; Marty, M. ; Vercellino, L. ; de Roquancourt, A. ; C Cuvier, C. ; F Coussy, F. ; Mpie, F. and Hindie, F. (2013). HER2-overexpressing breast cancer: FDG uptake after two cycles of chemotherapy predicts the outcome of neoadjuvant treatment. BRITISH JOURNAL OF CANCER .109.1157-1166

**Hoff ER,** Tubbs RR, Myles JL, Procop GW: HER2/neu amplification in breast cancer: stratification by tumor type and grade.Am J Clin Pathol 2002, 117:916-921

**Hawkins, RA**.; Roberts, MM. and Forrest, Ap.( 1980).Eestrogen receptors and breast cancer. Current status. Br J Surg . 67:162-165.

**Iraqi Cancer Registry Center Publications (**2009)**.** Ministry of Health, Iraqi Cancer Board. Baghdad, Iraq

**Joensuu**, **k. ,** Leidenius , M. ;Kero M. and Andersson L. C.( 2013). ER, PR, HER2, Ki-67 and K5 in Early and Late Relapsing Breast cancer—Reduced K5 Expression in Metastases. Basic and Clinical Research. 7 23–34.
**Jumaah, Sh. S. (2013).** Molecular Detection of Mutations in BRCA1, BRCA2, K-Ras and PIK3CA Genes in Iraqi Breast Cancer Patients and Their Families**.** PH.D. thesis. Baghdad University.pp.142.
**Khalifa** A el-A Helal T, , Kamel AS: Immunohistochemical expression of p53 and c-erbB2 proteins in breast cancer in Egypt. Anticancer Res 2000, 20:2145-2150
**Kruser, TJ**. and Wheeler, DL. (2010) . Mechanisms of resistance to HER2 family targeting antibodies. Exp Cell Res. 316:1083–100.

**Kumor, R.M.** (2009). The widely used diagnostic" DNA microarray" – a review . Am.J. Inf.Dis. 5:207-218.
**Kyndi, M**. ; Sørensen, F.; Knudsen, H. ; Overgaard, M. ; Nielsen, H.M. ;and Overgaard, J. (2008). Estrogen Receptor, Progesterone Receptor, HER-2, and Response to Postmastectomy Radiotherapy in High-Risk Breast Cancer: The Danish Breast Cancer Cooperative Group. JOURNALOF CLINICALONCOLOGY. 26. 9 . 1419 - 1426.
**Lemoine, N.** (1994). Molecular biology of breast cancer. Ann Oncol. 5:31-37.
**Luis, R.A**. (2012 ). Ottesen.M.E. Hughes. Impact of hormone receptor status on patterns of recurrence and clinical outcomes among patients with human epidermal growth factor-2-positive breast cancer in the National Comprehensive Cancer Network: a prospective cohort study. Breast Cancer Research . R129: 1-14.
**Moghimi**, **S.M.**; Rahbarizadeh, D.; Ahmad, V. and Parhamifar, L.( 2013). Heavy Chain Only Antibodies: A New Paradigm in Personalized HER2+ Breast Cancer Therapy. Bio Impacts. 3(1). 1-4 doi: 10.5681.
**Pauletti** G, Godolphin W, Press MF, Slamon DJ: Detection and quantitation of HER-2/neu gene amplification in human breast cancer archival material using fluorescence in situ hybridization. Oncogene 1996, 13:63-72
**Pauletti** G, Dandekar S, Rong H, Ramos L, Peng H, Seshadri R, Slamon DJ: Assessment of methods for tissue-based detectionof the HER-2/neu alteration in human breast cancer: adirect comparison of fluorescence in situ hybridization andimmunohistochemistry. J Clin Oncol 2000, 18:3651-3664.
**Penault-Llorca , F.** and Viale, G. ( 2012 ). Pathological and molecular diagnosis of triple-negative breast cancer: a clinical perspective . Annals of Oncology. 23 : 19–22.
**Saez, RA**. and Osborne ,CK. (1989) . Hormonal treatment of advanced breast cancer, in Kennedy BJ (ed): Breast Cancer. Current Clin. Oncol. New York.1. 163-172.
**Sørlie, T.** ; Perou, CM. and Tibshirani R. (2001). Gene expression patterns of breast carcinomas distinguish tumor subclasses with clinical implications. Proc Natl Acad Sci U S A. 98(19):10869–74.
**Slamon** DJ, Godolphin W, Jones LA, Holt JA, Wong SG, Keith DE,Levin WJ, Stuart SG, Udove J, Ullrich A, et al.: Studies of theHER-2/neu proto-oncogene in human breast and ovariancancer. Science 1989, 244:707-712.11
**Subik, K**. ; Lee, J. ; Baxter, L. ; Strzepek, T.; Costello, D. ; Crowley, P.; Xing, L. ; Hung, M. ; Bonfiglio, T. ; Hicks, D. and Tang, P.(2010). The Expression Patterns of ER, PR, HER2,CK5/6, EGFR, Ki-67 and AR by Immunohistochemical Analysis in Breast Cancer Cell Lines. Breast Cancer: Basic and Clinical Research. 4. 35–41.
**Taucher** S, Rudas M, Mader RM, Gnant M, Dubsky P, Bachleitner T, Roka S, Fitzal F, Kandioler D, Sporn E, et al.: Do we need HER-2/neu testing for all patients with primary breast carcinoma? Cancer 2003, 98:2547-2553.
**Rosenthal** SI, Depowski PL, Sheehan CE, Ross JS: Comparison of HER-2/neu oncogene amplification detected by fluorescence in situ hybridization in lobular and ductal breast cancer. Appl Immunohistochem Mol Morphol 2002, 10:40-46
**Varshney** D, Zhou YY, Geller SA, Alsabeh R: Determination ofHER-2 status and chromosome 17 polysomy in breast carcinomascomparing HercepTest and PathVysion FISH assay.Am J Clin Pathol 2004, 121:70-77.
**Yarden, Y**.( **2001**). Biology of HER2 and its importance in breast cancer. Oncology . 61 (Suppl 2):1–13.
**Young, S.L.** and Lessey, A.B.( 2010). Progesterone Function in Human Endometrium: Clinical Perspectives. 28(1):005-016.
**Yang** J, Xing T, Yao X, Hu R: Relationship of C-erbB-2 oncogene overexpression to estrogen progesterone receptor in breast cancer and its prognostic significance. Hua Xi Yi Ke Da Xue Xue Bao 1997, 28:214-217.
**Warford, A.;** Howat, W. and McCafferty, J. (2004). Expression profiling by high-throughput IHC. Journal of Immunological Methods. 290. 1(2) . 81-92.
**Wu,** S. C. ; Hotes, J. ; Fulton, J. P. ; Chen, V. W. ; Howe, H. L. and Correa, C . (2002) . Cancer in North America , 1995-1999. Volume III: Combined Cancer Incidence Rates. North American Association of Central Cancer Registries . Springfield. IL, U.S.A.

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