Evaluation of hormone receptors status (estrogen & progesterone) and human epidermal growth factor receptor R-2 (HER2) in breast cancer in Basrah

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ABSTRACT

Background: **Breast cancer is a complex disease that demands awareness by both physicians and patients. It is the most common type of malignancy among Iraqi women. Hormone receptors, Estrogen, Progesterone (ER and PR) and Human Epidermal Growth Factor Receptor-2 (HER-2) have a great influence on the clinical outcome of breast cancer. The role of hormone receptors and HER2 as a prognostic factor has a wide acceptance in the management of breast cancer.** ***Aim of the study:*** **To evaluate hormone receptor status in breast cancer cases in Basrah and to correlate them with other clinicopathological parameters of breast cancer.** ***Patients and methods:*** **Seventy six cases of breast cancer (mastectomy specimens) were collected. Formalin fixed and paraffin embedded (FFPE) histological sections stained by hematoxylene and eosin (H&E) and graded according to the Modified Nottingham grading system (NGS). Immunohistochemical (IHC) staining of FFPE sections and scoring for ER, PR and HER2 were done according to the DAKO Cytomation protocol using the Labelled Streptavidin Biotin system (LSAB).** ***Results:*** **The majority of patients were less than or equal to 50 years old (60.5%) with a mean age of 48 years. The leading tumor type is infiltrative ductal carcinoma (89.5%). The majority of patients are presented with grade II (72%) and stage IIA (40.8%). The percentage of ER +ve tumors is (55%) followed by PR +ve (48.7%) and HER2 +ve (30%). A significant positive correlation is found between ER, PR and age (P value 0.02) and a significant negative correlation between ER, PR and grade of tumor (P value 0.01). Also a negative correlation between ER, PR and stage of tumor is found, however this association is statistically not significant (P value 0.1). No significant association between HER2 with age and grade is found; but a significant positive relationship is shown between HER2 and stage of tumor (P value 0.01). An inverse correlation between ER and HER2 was just statistically significant (p value 0.05) while no significant association between PR and HER2. ER +ve/ PR +ve/HER2 –ve tumors (PPN) account for higher percentage among subgroups of breast cancer (30%) and frequently occur in the early grade and stage, followed by triple negative tumors (NNN) 19.7% which are mainly found in grade III and stage III tumor.** ***Conclusion:*** **breast cancer in our locality is characterized by an early age occurrence with a moderately advanced grade and stage at presentation. Hormonal status is nearly similar to that reported in other studies. ER +ve/ PR +ve (PPN) tumor is associated with earlier grade and stage than that of triple negative tumor (NNN). (MJBU,30,2: 2012, Page 133-142)**

أظهار حالة المستقبلات الهرمونية (استروجين، بروجستيرون) و مستقبل عامل النمو البشري للإنسان باستخدام الفحص المناعي النسيجي في حالات سرطان الثدي في البصرة

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الخلاصة: إن سرطان الثدي مرض معقد يتطلب الوعي المتبادل بين كل من الأطباء و المرضى. وهو من السرطانات الأكثر شيوعا بين النساء. ان المستقبلات الهرمونية (الاستروجين والبروجيستيرون) و مستقبل عامل النمو البشري عند الإنسان يشكلون تأثيرا كبيرا على الحصيلة السريرية لسرطان الثدي. وان دور هذه المستقبلات الهرمونية كعوامل انذرا لما يؤول إليه المرض له قبول واسع في معالجة سرطان الثدي. أهداف الدراسة: لتقييم حالة المستقبلات الهرمونية في مرضى سرطان الثدي و ربط ذلك مع المعايير المرضية السريرية الأخرى لسرطان الثدي. المواد والطرق: تم جمع ست وسبعين حالة من سرطان الثدي (عينات ثدي مستأصل) و تم فحص المقاطع النسيجية المثبتة في الفورمالين و المغروسة بإحكام في البارافين و المصبوغة بصبغة الهيماتوكسلين والايوسين و ثم درجت حسب نظام تدريج نوتنكهام المطور لسرطان الثدي. وقد اجري الصبغ المناعي النسيجي للمقاطع النسيجية باستعمال نظام (الساب) وتم تحريز المستقبلات الهرمونية المصبوغة. النتائج: معظم المرضى كانت أعمارهم 50 سنة أو اقل

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بنسبة (٦٠,٥ %) و بمعدل ٤٨ سنة. و كان النوع الرئيسي للمرض هو السرطان القنوي النافذ الارتشاحي (٨٩,٥%). و يظهر معظم المرضى في الدرجة الثانية للمرض بنسبة (٧٢%) ومرحلة المرض الثانية بنسبة (٨ ،٤٠%). كانت نسبة الأورام الموجبة لمستقبل الأستروجين (٥٥%)، تليه الأورام الموجبة لمستقبل البروجيستيرون (٤٨,٧%) ثم الموجب لمستقبل عامل النمو (٣٠%). وقد وجد إن هناك ارتباطا موجبا معتدا بين مستقبلات الاستروجين و البروجيستيرون مع العمر و ترابطا سالبا معتدا بين مستقبلات الاستروجين والبروجيستيرون مع درجة المرض. وهناك ترابط سلبي أيضا بين مستقبلات الاستروجين والبروجيستيرون مع مرحلة المرض ولكن هذا الترابط غير معتد إحصائيا. لايوجد ارتباط معتد بين مستقبل عامل النمو مع عمر المريض ودرجة المرض ولكن الارتباط الموجب المعتد كان بين مستقبل عامل النمو و مرحلة المرض. هناك ترابط عكسي كاد أن يكون معتدا بين مستقبل الاستروجين و مستقبل عامل النمو، بينما لايوجد ترابط بين مستقبل البروجيستيرون و مستقبل عامل النمو. إن الأورام الموجبة لمستقبلات الاستروجين و البروجيستيرون تشكل أعلى نسبة مئوية بين الأنواع الثانوية لسرطان الثدي (٣٠%) و هي كثيرة الوجود في درجة و مرحلة المرض المبكرة، تتبع بالأورام الثلاثية السالب (١٩,٧%) التي توجد بشكل رئيسي في درجة و مرحلة المرض الثالثة.الخاتمة: ان سرطان الثدي في منطقتنا يتميز بحدوثه في عمر مبكر مع درجة و مرحلة متقدمة عند الظهور.اظهرت الدراسة ان ما يقارب نصف المرضى كانوا ذو أورام موجبة مستقبلات الاستروجين أو البروجيستيرون و ثلث واحد كانت موجبة مستقبل عامل النمو البشري. الورم الموجب لمستقبلات الاستروجين و البروجيستيرون و السالب لمستقبل عامل النمو البشري يرتبط بدرجة و مرحلة المرض أبكر مما للورم الثلاثي السالب.

INTRODUCTION

Breast cancer is the most common cancer in women worldwide and the second leading cause of cancer death among females after lung cancer,[1] accounting for 23% of total cancer cases and 14% of cancer.[2] There is a wide variation in the incidence and mortality rate of breast cancer among populations, being highest in developed countries and lowest in developing countries.[3] This difference could be due the differences in the life style and reproductive habits or due to improved screening and diagnostic tools of breast cancer in developed countries**.** Breast cancer is the most common cancer among women in Arabic countries with a median age at presentation is around 50 years. There are increased percentage of younger-age group women with breast cancer which is associated with aggressive biological characters.[4] In Iraq, and according to the Iraqi Cancer Registry (ICR), breast cancer is accounting for 16.7% out of the total cancer cases and is the most common type of female malignancy accounting for 31.75% out of registered female cancer.[5] In Basrah, and according to Basrah Cancer Registry, breast cancer is also the most frequent cancer affecting female accounting for 30.2% of all cancer cases among females.[6] Virtually all breast cancers are adenocarcinoma, it is either non-invasive (limited to ductal or lobular epithelium with no penetration to basement membrane) or invasive ductal or lobular carcinoma with involvement of basement membrane.[7] Some breast cancers have hormone receptors such as estrogen and/or progesterone receptors (ER & PR). Tumor with hormone positive receptors has better prognosis than that with hormone negative receptors.[8] Some breast cancers have over-expression of Human Epidermal growth factor. Receptor 2(HER2) which is a product of an oncogene that helps cells to grow, divide and repair themselves. It is amplified and over-expressed in 20-30% of breast cancer. Breast cancer with HER2 over-expression tends to have more aggressive course, higher risk of recurrence and poor prognosis.[9] ER and PR status has been used for many years to determine a patient's suitability for endocrine therapy. More recently, testing for HER2 has been included in routine patient work-up, with recognition of its value both as a prognostic marker and, more particularly, in predicting response to trastuzumab (Herceptin).[10] The most widely

used breast cancer grading system is the modified Nottingham Grading System (NGS) of Scarff-Bloom Richardsom (SBR) system which depends on nuclear pleomorphism, tubular formation and mitotic rate.[11] A newer proposed semi-automated method for breast cancer grading (N+P) system used nuclear pleomorphism and proliferative index measured by immunohistochemistry.[12] Prognosis of breast cancer depends on many factors including age, histological type, histological grade and stage, hormone status and gene influence.[13]

Aim of the study, is to evaluate the expression of hormone receptors status (estrogen and progesterone) and HER2 over-expression in breast cancer cases in our locality and to compare them with patien. t's age, histological grade, and stage of tumor.

MATERIALS AND METHOD

This cross-sectional prospective study was carried out in the Department of Pathology and Forensic medicine, College of Medicine, University of Basrah during the period from November 2010 through September 2011. Seventy six cases of breast cancer (mastectomy specimens) were collected from Al-Sader teaching hospital and from private laboratories. All tissues are formalin fixed and paraffin embedded (FFPE). Two groups of sections of 5 mm thickness were taken from each FFPE sections. First group were stained by Hematoxylen and Eosin (H&E) staining, examined for histopathological type of tumor and graded according to modified Nottingham grading system (NGS). Second group were stained by immunohistochemistry according to Dakocytomation method of Labelled Streptavidine Biotin system (LSAB) by the use of primary antibodies for ER, PR and HER2. Scoring of ER and PR is done according to Allred scoring system as shown in (**Table-1)**. Immunohistochemical scoring of HER2 is shown in **(Table-2)**.

Table 1. Allred scoring system of ER and PR.

Proportion	Intensity score
0-1 1/100	0 not staining
2 1/10	1 weak
3 1/3	2 intermediate
4 2/3	3 strong
5 3/3	

Total score = proportion score + intensity score

Note: Total score for ER and PR more than 3 is considered as positive for ER and PR.

Table 2. IHC scoring system of HER2.

Score	Staining description	Significance
3+	Strong complete membrane staining in more than 30% of tumor cell population	Immuno positive
2+	Moderate membrane staining in more than 10% of tumor cell population	Weakly or border-line positive
1+	Either weak or perceptible membrane staining in more than 10% of tumor cell population	HER2 negative
Zero	Completely negative staining in less than 10% of tumor cell population	HER2 negative

RESULTS

The age groups ranged from 23 to 78 years old with a mean age of 48 years old. Most of the patients were less than or equal to 50 years old with a percentage of (60.5%). The leading tumor type was infiltrative ductal carcinoma (89.5%), and the other (10.5%) was diagnosed as lobular carcinoma. The majority of cases were of grade II with a percentage of 72%, followed by grade III (22.4%) and (5.3%) for grade I. The percentage of ER +ve breast cancer is (55%) followed by PR +ve (48.7%), and HER 2 over-expression is (30%). Stage IIA accounts

for the majority of cases (40.8%) followed by stage IIB (31.6%), then stage IIIA (17.1%) and finally stage IIIB (10.5%). During the period of the study no stage I or stage IV were recorded. As shown in **(Table-I).**

Figure 1 and 2: show the H&E staining characters of grade II and III respectively.
Figure 3, 4 and 5: show the IHC staining characters of ER, PR and HER2.

Table I. Patient age, tumor type, grade, stage and hormonal status percentages

Prognostic markers		No. of cases	%
Age	≤50	46	60.5
	>50	30	39.5
Type	Ductal	68	89.8
	Lobular	8	10.2
Grade	I	4	5.2
	II	55	72.4
	III	17	22.4
Stage	I	0	0
	IIA	31	40.8
	IIB	24	31.6
	IIIA	13	17.1
	IIIB	8	10.5
	IV	0	0
ER	+ve	42	55.3
	-ve	34	44.7
PR	+ve	37	48.7
	-ve	39	51.3
HER2	+ve	23	30.3
	- ve	53	69.7

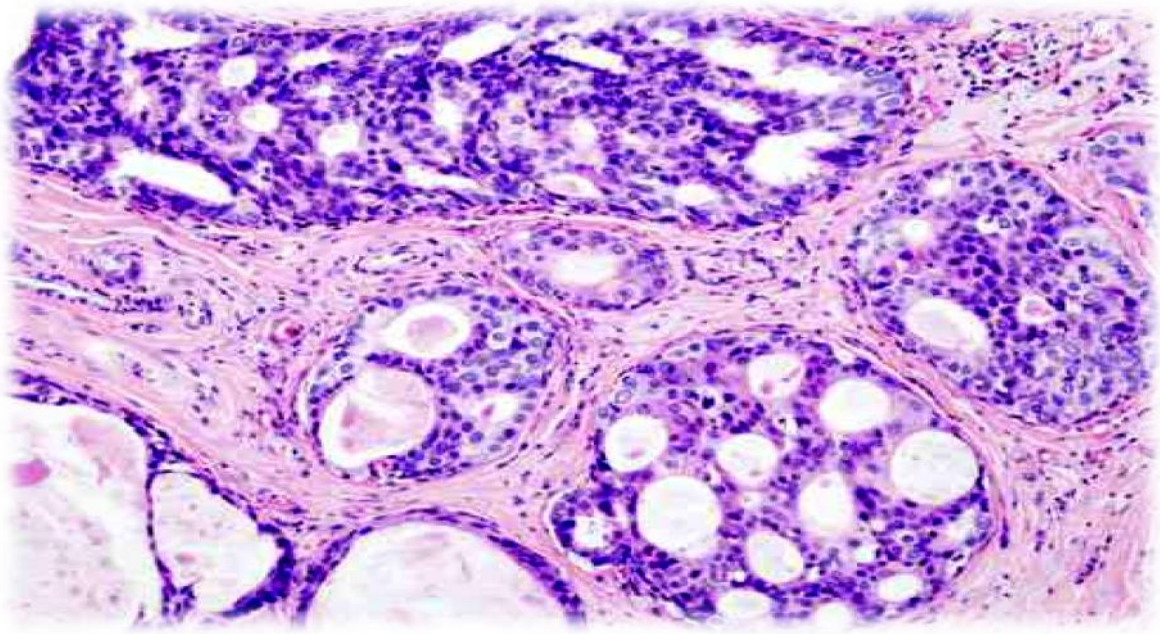

Fig1. H&E. Grade II breast cancer (100X).

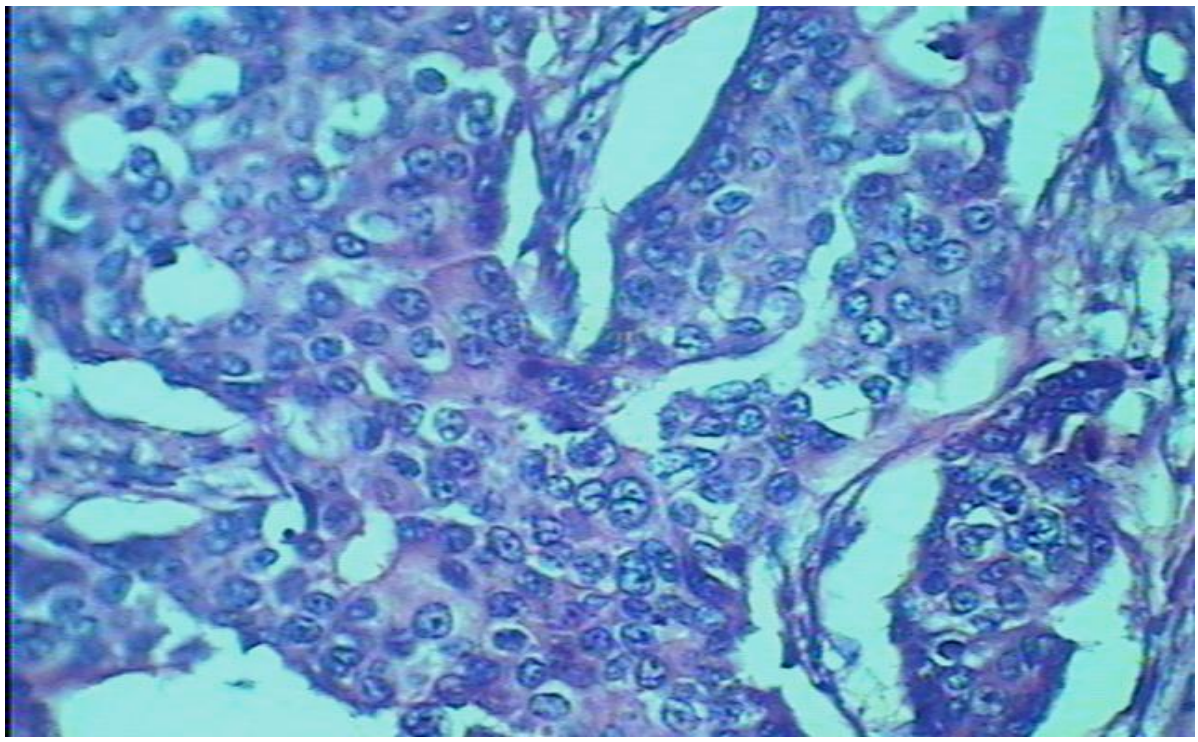

Fig 2. H&E. Grade III breast cancer (400X).

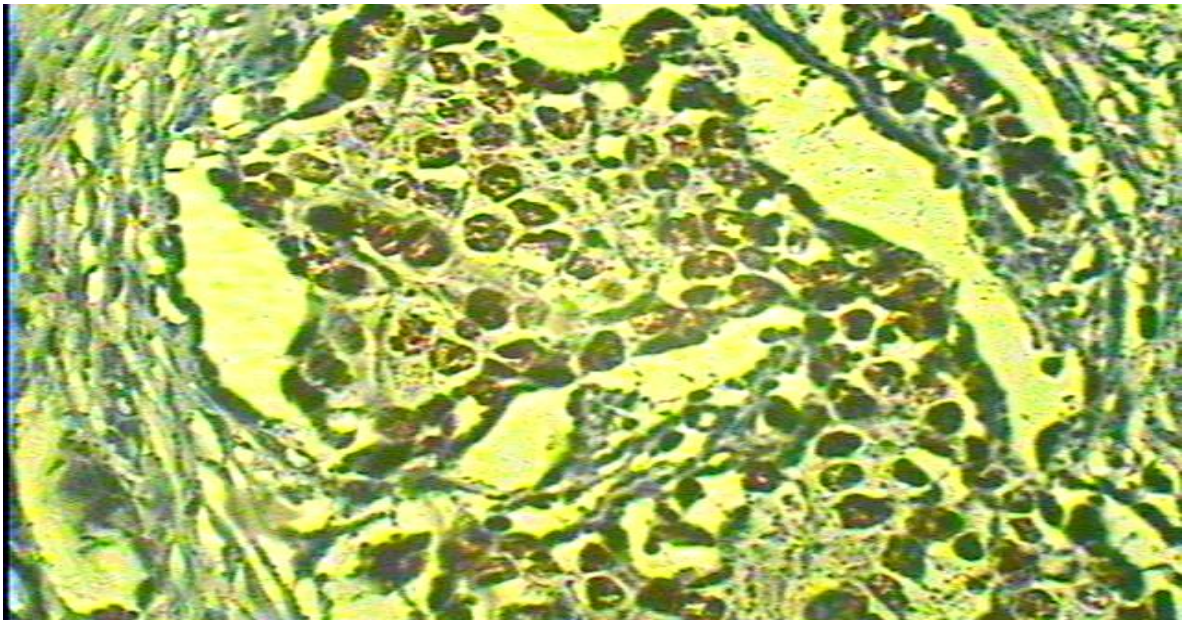

Fig 3. IHC staining. ER positive tumor cells in breast cancer (granular brownish discoloration of nucleus) 400X.

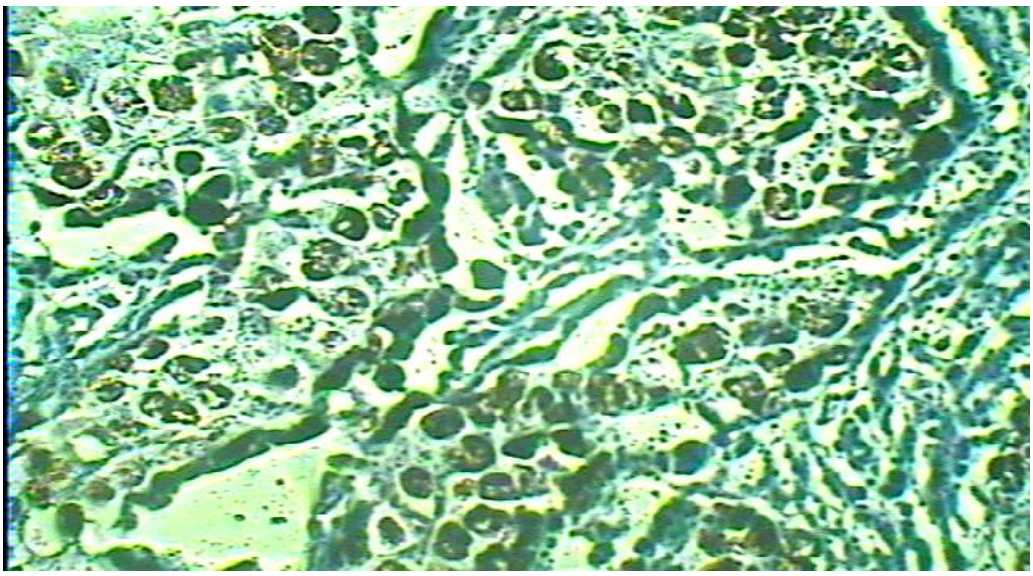

Fig 4. IHC staining. PR +ve tumor cells in breast cancer (granular brownish discoloration of nucleus) 400X.

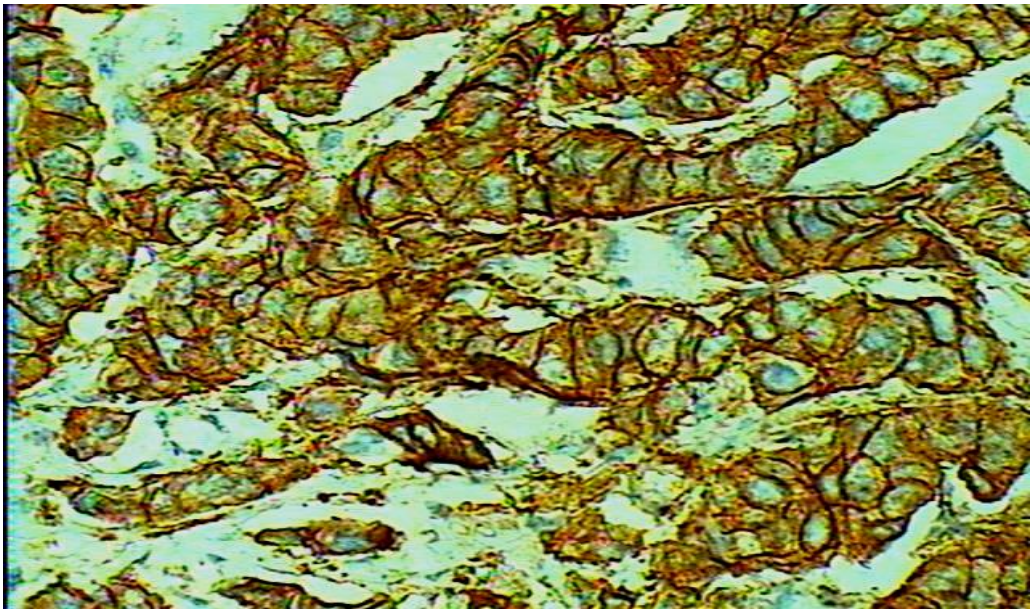

Fig 5. IHC staining. HER2 +ve strongly positive (3+) pattern showing intense complete membrane staining. 400X

There is a significant positive correlation between ER, PR and age (P value 0.02) and a significant negative correlation between ER, PR and grade of tumor (P value 0.01). Also a negative correlation between ER, PR and stage of tumor is found, however this association is statistically not significant (P value 0.1).There is No significant association between HER2 with age and grade; but a significant positive relationship between HER2 and stage of tumor is shown (P value 0.01) as shown in (Table-II). It has been found that there is an inverse correlation between ER and HER2 which is just statistically significant (p value 0.05) and majority of ER positive tumors were lacking HER2 (78.6%). Similarly the majority of PR +ve tumors (75.5%) were HER2 negative; however this association is not statistically significant (0.2) as shown in (Table-III). According to the positivity and negativity of ER, PR and HER2, the majority of cases were of ER +ve/PR +ve/HER2 –ve (PPN) 30%,

followed by triple negative tumor ER –ve/PR –ve/HER2-ve) (NNN) 19.7% as shown in (Table-IV). It has been showed that the majority of ER +ve/ PR +ve/HER2-ve tumors (PPN) frequently occur in older age group, lower grade and earlier stage. While triple negative tumors (NNN) mainly found in younger age patients, higher grade and later stage as shown in (Table-V).

Table II. The associations between ER, PR and HER2 with age, grade and stage of tumor.

Prognostic markers		No. of cases	ER		PR		HER2	
			+ve	-ve	+ve	-ve	+ve	-ve
Age	≤50	46	19 (41.3%)	27 (58.7%)	18 (39%)	28 (61%)	16 (34.8%)	30 (65.2%)
	>50	30	23 (76.7 %)	7 (23.3%)	19 (63.3%)	11 (36.7%)	7 (23.3%)	23 (76.7%)
Total		76	42	34	37	39	23	53
P value			0.02		0.03		0.3	
Grade	I	4	4 (100%)	0	4 (100%)	0	1 (25%)	3 (75%)
	II	55	35 (63.6%)	20 (36.4%)	31 (56.4%)	24 (43.6%)	13 (23.6%)	42 (76.4%)
	III	17	3 (17.6%)	14 (82.4 %)	2 (11.8%)	15 (88.2%)	9 (53%)	8 (47%)
Total		76	42	34	37	39	23	53
P value			0.01		0.01		0.06	
Stage	IIA	31	23 (74.2 %)	8 (25.8%)	24 (77.4%)	7 (22.6%)	5 (16%0	26 (84%)
	IIB	24	13 (54.2%)	11 (45.8%)	7 (29.2%)	17 (70.8%	6 (25%)	18 (75%)
	IIIA	13	4 (30.8%)	9 (69.2%)	4 (30.8)	9 (69.2%)	5 (38.5%)	8 (61.5%)
	IIIB	8	2 (25%)	6 (75%)	2 (25%)	6 (75%)	7 (87.5%)	1 (12.5%)
Total		76	42	34	37	39	23	53
P value			0.1		0.1		0.01	

Table III. Association between ER, PR and HER2.

Hormone receptors		No. of cases	Her2		P value
			+ve	-ve	
ER	+ve	42	9(21.4%)	33(78.6%)	0.05
	-ve	34	14(41.2%)	20(58.8%)	
PR	+ve	37	9 (24.3%)	28 (75.7%)	0.2
	-ve	39	14 (35.9%)	25 (64.1%)	
Total		**76**	**23 (30.3%)**	**53 (69.7%)**	

Table IV. The percentages of tumor cases according to the positivity and negativity of ER, PR and HER2.

No. of cases (%)	ER	PR	HER 2
5 (6.6)	+	+	+
23 (30.2)	+	+	-
11(14.5)	+	-	-
3 (3.9)	+	-	+
5 (6.6)	-	+	+
4 (5.3)	-	+	-
10(13.2)	-	-	+
15 (19.7)	-	-	-

Table V. The association between subgroups of breast cancer with age, grade and stage of tumor.

Tumor Sub-Groups	No. of cases	Age		Grade			Stage			
		≤ 50	> 50	I	II	III	IIA	IIB	IIIA	IIIB
PPN	**23**	7 15.2%	16 53.3%	3 75%	17 31%	3 17.6%	13 42%	6 25%	3 23%	1 12.5%
NNN	**15**	14 30 %	1 3.3%	0	6 10.9%	9 52.9%	2 6.4%	4 16.6%	6 46.2%	3 37.5%
PNN	**11**	3 6.5%	8 26.6%	1 25%	9 16%	1 5.8%	3 9.6%	7 29%	1 7.7%	0
NNP	**10**	8 17.3%	2 6.6%	0	6 11%	4 23.5%	1 3.2%	3 12.5%	3 23%	3 37.5%
NPP	**5**	4 43.4%	1 16.6%	0	5 45.4%	0	3 48.3%	2 41.6%	0	0
PPP	**5**	4 43.4%	1 16.6%	0	5 \45.4%	0	4 64.5%	1 20.8%	0	0
NPN	**4**	3 26.6%	1 13.3%	0	4 29.09%	0	3 38.7%	1 16.6%	0	0
PNP	**3**	3 6.6%	0	0	3 16.3%	0	2 19.3%	0	0	1 37.5%
Total	**76**	**46**	**30**	**4**	**55**	**17**	**31**	**24**	**13**	**8**

Note: The arrangement of receptors is ER, PR, and HER2
P= positive, N= negative

DISCUSSION

Breast cancer is a disease with a tremendous heterogeneity in its behavior. Clinical and pathological variables of tumors such as histological grade, type, stage, age and hormonal status may help in predicting prognosis and need for adjuvant therapy.[14,15] In this study, breast cancer is found to affect younger age women. This is corresponding to similar studies in other parts of Iraq,[16,17] some Arabic countries,[14,15,18,19-21] Iran [22] and third world countries.[23,24,25,26] However in western countries the disease is more common in older age women.[27,28] These finding could be due to environmental or genetic factors. It has been found that the majority of the patients (72%) presented with grade II and stage II cancer. These findings have been reported in other studies.[4, 15- 20, 29-31] This late presentation may reflect the poor health education of general population and ignorance regarding the significance of clinical breast examination, breast self examination, early medical consultation and screening programme. Also negligence, poverty and fear of mastectomy play an important role in delay presentation. The prevalence of ER & PR positivity was 55% and 49% respectively. The finding of ER expression is nearly similar to results of other studies done in Baghdad, [16] Jordon[14] Saudi Arabia [15] Tunis[18] Egypt, [19] which range from 53-65%. And differ from those reported in Lebanon[22] black women in US and Austrian women[32] Nigeria.[33] Variation in the percentage of ER expression among population could be attributed to factors related to tissue fixation and antigen preservation, because prolonged tissue fixation (more than 24 hours) can cause masking of the antigenic epitope and results in strong non-specific background staining.[10] The percentage of HER2 over-expression was 30% which appear to be within higher limit of accepted rates of 20-30%.[9] This result is nearly similar to what reported in studies done in Jordon[14] Saudi Arabia[15] Tunis[18] and Egypt[19] But it differ from study done in Baghdad [16] and Iran [23] which show higher percentage of HER2 over-expression (46%) This variation in HER2 expression may

reflect differences in the subjective evaluation of HER2 status. An inverse association has been found between HER2 over-expression and the presence of receptors for estrogen and progesterone; thus the higher the level of HER2 over-expression the lower the corresponding ER and PR positivity. These finding are in agreement with results in other worldwide studies.[14,15,17,18,26,27] This has been explained by hormone–dependent down regulation of HER2 involving a complex molecular interaction. Estrogen and its receptor are required to suppress HER2, [15] this explained the HER2 over-expression in women with low or absent ER expression. But they differ from study in Iran[23] which shows no significant association between hormone receptors and HER2 status. A strong association has been found between patient age and ER, PR expression. Younger patients are more likely having breast cancer with negative hormone receptors than older women. These findings are similar to that found in other studies.[14,16,15,18,19] No significance association has been found between HER2 over-expression and age of the patients. These finding differ from the results of other studies which show a significant negative association between age and HER2 over-expression[16,14,18, 23] and also differ from results of study done in Saudi Arabia[15] which showed that HER2 over-expression was significantly higher among post menopausal women. But these results were similar to study done in Egypt[19] which also shows that there is no significant association between HER2 over-expression and age of the patient. These differences could be also due to the subjective nature of HER2 scoring system. A significant association has been found between tumor grade and ER, PR expressions; the lower the grade of tumors the higher the ER and PR positivity and vice versa. Therefore, there is a uniform loss of ER content as the tumor becomes more anaplastic indicating that hormone receptors status could represent one aspect of tumor cell differentiation, as the grade increase (cell differentiation decrease) and hormone expression decrease. These findings are similar to other studies.[14-16,18,19] In this study, it has been found that HER2 over-expression was positively correlated with tumor grade and 53% of grade III tumors were HER2 +ve, but this finding was not statistically significant. This result is similar to that reported in other studies.[14-18] which show no significant association between HER2 and grade of tumor. However, these results differ from other studies which show significant association between HER2 and histological grade.[14,19] This difference could be due the small sample size. An inverse relationship has been found between hormone receptors ER & PR positivity and stage of tumor i.e. the percentage of ER+ve and PR+ve tumors decrease as the stage increase; but this association is not statistically significant, these findings were similar to other studies.[18,23] This can be explained by the direct association between grade and stage of tumor. However the small sample size may be responsible for the insignificant relationship between hormone receptors and tumor stage. There is a significant direct association between HER2 and stage of tumor. These findings are corresponding with results in other studies.[14-16,18,33,34] But it differs from study done in Iran which shows no significant association between HER2 and stage of tumor.[23] In this study, it has been found that tumor with positive ER and PR and negative HER2 (PPN) formed the higher percentage among other types of breast cancer (30%) and is associated with older age women, lower grade and earlier stage (better prognosis), followed by tumor with triple negative receptors (NNN) 19.7% which commonly seen in younger age women, grade III and stage IIIA at presentation (worse prognosis). These finding are corresponding to the results of other studies. [15, 35]

In conclusion, breast cancer in our locality is characterized by an early age occurrence. Most patients regardless to their age are presented with a moderately advanced grade and stage at

time of diagnosis. About half of the patients had positive hormonal expression (ER or PR) and one third had HER2 over-expression. There is a negative correlation between HER2 over-expression and hormone receptors. ER+ve/PR+ve/HER2 –ve tumors are occurred frequently in older women and are associated with more favorable clinico-pathological parameters than that of TNBC (Triple Negative Breast Cancer).

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