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Original Research Article

Immunohistochemical Evaluation of Triple Markers (ER, PR and HER-2/neu) in Carcinoma of the Breast in the North of Iraq.

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Objective: **The aim of this study is to evaluate the patterns of expression of ER and PR and HER-2 /neu over expression in carcinoma of the breast in Nineveh Province (north of Iraq), to correlate the expression of the triple markers with other prognostic parameters and to compare the result of this study with those of others.** ***Patient and Methods:*** **A cross sectional study of 128 female breast cancers, were collected in Nineveh Province (north of Iraq), from Jan.2014 to Jan. 2015. The expression of the triple markers were assessed immunohistochemically on formalin fixed paraffin embedded tissue of tumor. The pattern of expression of the triple markers was divided into four groups. The results of these were correlated with age, tumor size, types and grade and lymph node status.** ***Results:*** **Each of hormonal receptors (ER and PR) were expressed in 63.3% of the cases, while HER-2/neu over expression was found in 18% of the cases. The pattern of expression of ER+, PR +and HER-2 /neu-ve was found in 56.3% of the cases. While the triple immunonegative pattern was observed in 25.8% of the cases. The mean age of patients with positive ER and PR expression was 57.4 years as opposed to 45.7 years among patients lacking hormonal expression. The mean age of patients with positive HER-2/neu was 48 years, which is 10 years younger than those who lack HER-2/neu over expression. The mean size of tumors expressing both ER and PR was 3.7 cm versus 5.2 cm for those lacking ER and PR expression. Tumors with strong HER-2/neu over expression tended to be larger than those lacking over expression, with mean sizes of 4.9 cm and 4.3cm respectively. An inverse relationship between hormonal receptors expression and the grading of the tumor. While there is a direct relationship between HER-2 over expression and the grading of the tumor. No statistical significant association was detected between both hormone receptors and HER-2 /neu positive cases on one hand and lymph node metastasis on the other hand.** ***Conclusions:*** **HER-2/neu over expression was found in 18% of breast carcinoma. This over expression is associated with young age at presentation, large tumor size, high histological grade and lack of ER and PR expression. In contrast, each of hormonal receptors (ER and PR) were expressed in 63.3% of the cases. The triple immunonegative pattern was observed in 25.8% of the cases. The pattern of expression of the triple markers was statistically significant correlate with histopathological typing, grading and lymph node status.**

Keywords: Breast carcinoma, hormone receptors, HER-2/neu, triple markers, immunohistochemistry.

INTRODUCTION

For many years strategies for the treatment and prognosis of breast cancer depends upon numerous factors such as patient age, tumor size, grade and stage of the tumor, the number of lymph nodes involved, and the presence of metastasis[(1-3)]. In the last decades immunohistochemical detection of ER, PR, HER-2/neu and other markers have prognostic and therapeutic significance[(3-9)]. Therefore, therapy is individualized depending on the pattern of expression of these markers [(4-7)].

Many studies have suggested that, the clinical and pathologic response to both hormone and chemotherapy varies with the pattern of expression of ER, PR and HER-2 subtypes [(8-19).] In Iraq, according to the Ministry of Health / Iraqi cancer registry 2014, breast cancer was the most frequent cancer among the women and accounts for approximately one-third of the registered female cancers, which shows a trend for the disease to affect younger women [(20)].

PATIENTS AND METHODS

This is a cross sectional study. Samples of primary carcinoma of breast, obtained from consecutive mastectomies of 128 female patients, were collected from Al-Jumhuri Teaching Hospital and some private labs. in Nineveh Province (north of Iraq). Tissue blocks were collected during a period extending from Jan. 2014 to Jan. 2015.

For each case, all Hematoxyline and Eosin stained sections were reviewed concerning the type, grade, and axillary lymph node status. The tumors were typed according to the WHO classification system and graded according to the

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Nottingham modification of Bloom-Richardson grading scheme [21].The size of the tumor mass was defined by gross pathological measurements as maximum diameter and divided into three groups according to TNM staging system [22].The pattern of expression of the triple markers were divided into four groups, these are:

1. ER +,PR+ /HER-2 –ve
2. ER+, PR+ /HER-2 +
3. ER-ve, PR-ve /HER-2 +, and
4. Triple immunonegative (ER-ve, PR-ve/HER-2 -ve).

Slides for immunohistochemistry were stained for ER, PR, and HER2/neu using antibodies, buffers and linking systems purchased from DAKOTM (Dako, Denmark). ER used clone SP1, PR clone Pg R 636, and HER2/neu polyclonal rabbit anti-human c-erbB- 2.Immunohistochemistry was performed according to manufacturer instructions.

Positive and negative control slides were involved in each run of staining. A Positive control slides for ER and PR were prepared from breast carcinoma known to be positive for the hormone receptors studied. Moreover, the normal breast ductal epithelial cells act as an internal positive control too. While negative control slides were prepared from the same tissue block, but incubated with TBS instead of the primary antibody. The staining pattern for HER2/neu is confined to the cell membrane. The staining pattern was compared with the control slides provided by the company.

A semi-quantitative score was used to record results of staining pattern for ER and PR which is confined to the nucleus according to the system established by Allred et al. [23]. This system considers both the proportion and the intensity of stained cells. 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 intensity score (IS) ranges from 0 to 3, with 0 being no staining, 1 weak staining, 2 intermediate staining, and 3 intense staining. 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 over expression was assessed according to Hercep Test TM Guidelines for scoring of HER2/neu [24]. Which was scored from 0 to 3 scale. 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. 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/neu positive cases. Fluorescence in situ hybridization(FISH) was not performed on the weak positive cases (score 2) in this study, due to limited facility in our locality.

Data was analyzed by using the X^2-test, the Student's t-test, or Fisher Freeman Holton's when necessary. The results were statistically considered significant if the p value was <0.05.

RESULTS

One hundred and twenty eight mastectomy specimens from patients with carcinoma of the breast were studied concerning the pattern of expression of the triple markers and their relation to other prognostic parameters. Details about the age of patients, size of the tumor mass, histopathological types and grad for breast carcinoma and the lymph node status ,and their relation to ER&PR expression and HER-2/neu over expression were clarified in table (1).

The distribution of the patterns of expression of the triple markers in the sampled patients with carcinoma of the breast

Each of hormonal receptors (ER and PR) were expressed in 81/128 (63.3%) of the cases, while HER-2/neu over expression was found in 23/128 (18%) of the cases. An inverse relationship between them was noted with p value 0.002, as shown in table (1).

The distribution of the pattern of expression of these triple markers in the present study was as follows; ER+/PR +and HER-2 /neu-ve was found in 72 (56.3%) cases of breast carcinoma. While the triple immunonegative pattern was observed in 33 (25.8%) cases, 14 (10.9%) cases were expressed the pattern ER-ve/PR -ve and HER -2 neu +ve, while 9 (7%) cases only were expressed the pattern of ER+/PR +and HER-2 /neu+ve.

Relation between the pattern of expression of the triple markers & the age of patients with of carcinoma of the breast

The mean age of patients with positive ER and PR expression was 57.4 years as opposed to 45.7 years among patients lacking hormonal expression with P value 0.002. For patients less than 50 years of age 32/47 (68.1%) were ER and PR negative, whereas 43/81 (53.1%) of patients 50 years or older were ER and PR positive with P value 0.008. The mean age of patients with positive HER-2/neu over expression was 48 years, which is 10 years younger than those who lack HER-2/neu over expression.

This difference is statistically significant with P value 0.006. Similarly, HER-2/neu over expression was seen in 13/23 (56.5%) of patients less than 50 years of age as opposed to 10/23 (43.5%) in patients 50 years or older with P value 0.004, as shown in table (1). The pattern of expression of the triple markers failed to show statistically significant association with the age of patients, with P value 0.28, as shown in table (2).

Relationship between the pattern of expression of the triple markers and the size of tumor mass in patients with carcinoma of the breast

The mean size of tumors expressing ER and PR was 3.7 cm versus 5.2 cm for those lacking both ER and PR expression with P value 0.007. ER and PR positive tumors comprised 63/81(77.8%) of tumors in group T2, but only 9/81 (11.11%) of those in group T3 with P value 0.06. Tumors with strong HER-2/neu over expression tended to be larger than those lacking over expression, with mean sizes of 4.9 cm and 4.3 cm, respectively. Among patients in groupT3, 6/23 (26.1%) were HER-2/neu positive compared to 12/23 (52.2%) with tumors in group T2 with P value 0.15, as shown in table (1).

The pattern of expression of the triple markers failed to show statistically significant association with the size of tumor

mass in patient with carcinoma of the breast, with P value 0.16, as shown in table (3).

Relationship between the pattern of expression of the triple markers and the histopathological types of carcinoma of the breast

Hormonal receptors were expressed in 67/81(82.7%) of the invasive duct carcinoma cases compared to 13/81(16.1%) of lobular carcinoma cases. HER-2/neu was over expressed in 21/23 (91.3%) of the invasive duct carcinoma cases compared to only 2/23 (8.6%) of our lobular carcinoma cases. None of the other types of breast carcinoma showed evidence of HER-2/neu over expression, as shown in table (1) and figures (1, 2,3 and 4). Statistically significant association was found between the pattern of expression of the triple markers and the types of carcinoma of the breast, with P value 0.003, as shown in table (4).

Relationship between the pattern of expression of the triple markers and the grade of invasive carcinoma of the breast

An inverse relationship between hormonal receptors expression and the grading of the tumor with P value 0.003 was seen. While there is a direct relationship between HER-2/neu over expression and the grading of the tumor with P value 0.04, as shown in table (1).
There was a significant relation between the pattern of expression of the triple markers and the grade of invasive carcinoma of the breast, with P value 0.001, as shown in table (5).

Relationship between the pattern of expression of the triple markers and the lymph node status in patients with carcinoma of the breast

Hormone receptors positive cases was found among those with up to three lymph node metastases in 26/81 (32.1%) cases, lower than the 32/81 (39.5%) cases seen among those with more than three lymph node metastases, but this difference was not statistically significant with P value 0.31.

HER-2/neu over expression was found in 12/23 (52.2%) cases who had more than three lymph node metastases, as opposed to 40/105 (38.1%) among the HER-2/neu negative cases with P value 0.29, as shown in table (1). Statistically significant association was found between the pattern of expression of the triple markers and the lymph node status in patients with breast carcinoma, with P value 0.004, as shown in table (6).

DISCUSSION

The IHC assessment of the pattern of expression of the triple markers in tumor tissue is an important parameters in the evaluation of carcinoma of the breast. Although hormonal status provides prognostic information, currently the major clinical value is the identification of the pattern of the triple markers in carcinoma of breast, which has led to a rational basis for many observations concerning the predicting prognosis and the response of advanced and recurrent tumors or resistant to traditional systemic therapy and the potential use of newer drugs such as trastuzumab in the case of HER-2/neu over expression[4,25,26].

Moreover, it has been reported that carcinoma of breast that expressed ER and PR, while not over express HER-2/ neu behave better, both clinically and biologically than breast carcinoma that do not express ER and PR, while over express HER-2/neu [1-11,25-28].

The distribution of the patterns of expression of the triple markers in carcinoma of the breast of the sampled patients

In the present study, a strong correlation between ER and PR expression was noted, each of hormonal receptors (ER and PR) were expressed in 63.3% of the sampled patients. These expressions were relatively consistent with the reported ranges in different studies, as shown in table (*i*), which indicate that the majority of the breast carcinoma in the study sample would respond to endocrine therapy.

In the current study HER-2/neu over expression was found in 18% of the sampled patients. The relatively lower HER-2/neu over expression in the present study were relatively consistent with the lower reported ranges in different studies as shown in table (*ii*). This variation may be related to some factors like 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. Moreover, this variation in HER-2/neu over expression may reflect differences in subjective evaluation of HER-2/neu status.

In the current study, an inverse relationship between hormone receptors expression and HER-2/neu over expression was noted with p value 0.002. Nearly all investigators report a negative relationship between these markers [1,8,11,13,14,16,25-30]. But they differ from study in Iran [12] which shows no significant association between hormone receptors and HER-2/neu status. This inverse association has been linked to the fact that estrogens and it's receptor are required to suppress HER-2/neu[1,8,13,16,25-30]. This leads to lower or absent hormone receptors in women with HER-2/neu positive breast cancers. This is one of the reasons why women who express HER-2/neu may be resistant to tamoxifen [2,4,25,26,32].

In the current study, the results of the pattern of expression of the triple markers are approximately more or less within the rate of detection of those found in many other studies as shown in table (*iii*).

This wide range of detection of hormone receptors expression, HER-2/neu over expression and the pattern of expression of triple markers in different studies may be attributed to the number of cases studies, IHC methodology used including tissue fixation, choice of antibody, sensitivity of the detection system and the determination of criteria for positive results used. Also differences in population groups, diversity of risk habits and variation of genetic predisposition may contribute to that wide range of variation in the pattern of expression of the triple markers that were reported in different countries [1-19,25-31].

It is observed in literatures that immunonegative breast carcinoma carries poor prognosis and is not amenable to both hormone therapy and chemotherapy [1,5,9,12,14,16,17,27,35,36]. In the current study, on analyzing the triple immunonegative pattern which was found in 25.8% of the cases, of these triple immunonegative cases 66.7% were seen under the age of 50 years, 60.6% with tumor size 2 -5 cm, 60.6% of IDC type, while 21.2% of ILC type, 48.5% of tumors were grade 11 and 51.5% of tumors were in grade 111 and 75.8% cases showing lymph node metastasis indicating bad prognosis.

Table 1: Correlation of ER and PR expression, HER-2 /neu over expression with other prognostic parameters.

Prognostic parameters	No.(%)	ER&PR Positive No. (%)	ER&PR Negative No. (%)	HER-2Positive No. (%)	HER-2 Negative No. (%)
Age					
20-29	3 (2.3%)	1 (1.2%)	2 (4.3%)	0 (0%)	3 (2.9%)
30-39	20 (15.6%)	11(13.6%)	9 (19.1%)	5 (21.7%)	15 (14.3%)
40-49	**47 (36.7%)**	26(32.1%)	21(44.7%)	8 (34.8%)	39 (37.1%)
Total less than 50	**70(54.7%)**	**38(46.9%)**	**32(68.1%)**	**13(56.5%)**	**57(54.3%)**
50-59	37 (29%)	28(34.6%)	9 (19.1%)	7 (30.5%)	30 (28.6%)
60-69	17 (13.3%)	13(16.1%)	4 (8.5%)	3 (13%)	14 (13.3%)
>70	4 (3.1%)	2 (2.5%)	2(4.3%)	0 (0%)	4 (3.8%)
Total 50 & older	**58(45.3%)**	**43(53.1%)**	**15(31.9%)**	**10(43.5%)**	**48(45.7%)**
Mass size					
< 2cm	17 (13.3%)	9 (11.1%)	8 (17.1%)	5(21.7%)	12(11.4%)
2-5cm	**93(72.7%)**	63(77.8%)	30(63.8%)	12(52.2%)	81(77.2%)
>5cm	18 (14%)	9 (11.1%)	9 (19.1%)	6 (26.1%)	12(11.4%)
Histological type					
IDC	101(78.9%)	67(82.7%)	34(72.3%)	21(91.3%)	80(76.2%)
ILC	20 (15.6%)	13(16.1%)	7 (14.9%)	2 (8.7%)	18(17.1%)
Medullary	3 (2.3%)	1 (1.2%)	2 (4.3%)	0 (0%)	3 (2.8%)
Mucinous	2 (1.6%)	0 (0%)	2 (4.3%)	0 (0%)	2 (1.9%)
IDC+Neuroendocrine	1 (0.8%)	0 (0%)	1 (2.1%)	0 (0%)	1 (1%)
Metaplastic	1 (0.8%)	0 (0%)	1 (2.1%)	0 (0%)	1 (1%)
Histological Grade					
I	1 (0.8%)	1 (1.2%)	0 (0%)	0 (0%)	1 (1%)
II	**71 (55.5)**	49(60.5%)	22(46.8%)	10(43.5%)	61(58.1%)
III	56 (43.7)	31(38.3%)	25(53.2%)	13(56.5%)	43(40.9%)
LN status					
NO LN metastasis	34 (26.6%)	23(28.4%)	11(23.4%)	5(21.7%)	29(27.6%)
LN metastasis	**94 (73.4%)**	58(71.6%)	36(76.6%)	18(78.3%)	76(72.4%)
1-3	42 (32.8%)	26(32.1%)	16(34%)	6(26.1%)	36(34.3%)
4-9	44 (34.4%)	27(33.3%)	17(36.2%)	10(43.5%)	34(32.4%)
≥10	8 (6.2%)	5 (6.2%)	3 (6.4%)	2 (8.7%)	6 (5.7%)
Total	**128(100%)**	**81(100%)**	**47(100%)**	**23(100%)**	**105(100%)**

Relationship between the pattern of expression of the triple markers and the age of patients with carcinoma of the breast

In the current study, 54.7% of patients were less than 50 years old and 45.3% of them were 50 years and more. In contrast to what is commonly known about a rising incidence of BC with age. The mean age of our patients was 49.1 years. The result of the present study revealed that mean age and the range of age of the sampled patients were approximately more or less within those of others, as shown in table (*iv*).

Numerous theories have been proposed to explain this variation in both the mean and the range of age of the patient with BC, including age of menarche, time of first delivery, parity, socio-demographic factors, genetic difference, traditional marriages among first-degree relatives, which are very common in Iraq and, accordingly, hereditary factors could play a major role. Another factor could be the degree of obesity associated with a diet high in fat, carbohydrate, and protein, and lack of exercise, which have been prevalent in Iraq. These are not completely satisfactory and more researches are needed in this locality to determine the predisposing factors in our patients.

The results of this study could explain the effect of hormonal status in female patient that play an important role in the behavior of this cancer. In the present study, there is a direct association between patient age and hormones receptors expression.

Table 2: Relationship between the pattern of expression of the triple markers and the age of patients with carcinoma of the breast. P. value (0.28) N.S.

Age	Total No.	%	The Pattern ER+,PR+ &HER-2-ve		The pattern ER+,PR+ & HER-2+		The pattern ER-,PR-& HER-2+		The Pattern ER-,PR-& HER-2-ve	
			No.	%	No.	%	No.	%	No.	%
20-29	3	2.3	1	1.4	0	0	0	0	2	6.1
30-39	20	15.6	11	15.3	0	0	5	35.7	4	12.1
40-49	47	36.7	23	31.9	3	33.3	5	35.7	16	48.5
Total less than 50	70	54.6	35	48.6	3	33.3	10	71.4	22	66.7
50-59	37	29	24	33.4	4	44.4	3	21.5	6	18.1
60-69	17	13.3	11	15.3	2	22.3	1	7.1	3	9.1
>70	4	3.1	2	2.7	0	0	0	0	2	6.1
Total 50 & older	58	45.4	37	50.4	6	66.7	4	28.6	11	33.3
Total	128	100	72	100	9	100	14	100	33	100

Table 3: Relationship between the pattern of expression of the triple markers and the size of the tumor mass in patients with carcinoma of the breast. P. value (0.16) N.S.

Mass size (cm.)	Total No.	%	The Pattern ER+,PR+ &HER-2-ve		The Pattern ER+,PR+ &HER-2+		The Pattern ER-,PR-& HER-2+		The Pattern ER-,PR-& HER-2-ve	
			No.	%	No.	%	No.	%	No.	%
<2Cm.	17	13.3	5	7	**4**	**44.5**	1	7.1	7	21.2
2 - 5Cm.	**93**	**72.7**	**61**	**84.7**	2	22.2	**10**	**71.4**	**20**	**60.6**
>5 Cm.	18	14	6	8.3	3	33.3	3	21.5	6	18.2
Total	128	100	72	100	9	100	14	100	33	100

Table 4: Relationship between the pattern of expression of the triple markers and the histopathological types of carcinoma of the breast. P. value (0.003).

Histological type	Total No.	%	The Pattern ER+,PR+& HER-2-ve		The Pattern ER+,PR+& HER-2+		The Pattern ER-,PR- & HER-2+		The Pattern ER-,PR- &HER-2-ve	
			No.	%	No.	%	No.	%	No.	%
IDC	**101**	**78.9**	**60**	**83.3**	**7**	**77.8**	**14**	**100**	**20**	**60.6**
ILC	20	15.6	11	15.3	2	22.2	0	0	7	21.2
MEDULLARY	3	2.3	1	1.4	0	0	0	0	2	6.1
MUCINOUS	2	1.6	0	0	0	0	0	0	2	6.1
IDC+NEUROENDOCRINE	1	0.8	0	0	0	0	0	0	1	3
METAPLASTIC	1	0.8	0	0	0	0	0	0	1	3
Total	128	100	72	100	9	100	14	100	33	100

Table 5: Relationship between the pattern of expression of the triple markers and the grade of invasive carcinoma of the breast. P. value (0.001) significant.

Grade	Total No.	%	The Pattern ER+,PR+& HER-2-ve		The Pattern ER+,PR+& HER-2+		The Pattern ER-,PR-& HER-2+		The Pattern ER-,PR-& HER-2-ve	
			No.	%	No.	%	No.	%	No.	%
I	1	0.8	1	1.4	0	0	0	0	0	0
II	**71**	**55.5**	**45**	**62.5**	4	44.4	6	42.9	16	48.5
III	56	43.7	26	36.1	**5**	**55.6**	**8**	**57.1**	**17**	**51.5**
Total	128	100	72	100	9	100	14	100	33	100

Table 6: Relationship between the pattern of expression of the triple markers and the lymph node status of patients with carcinoma of the breast. P. value (0.004) significant.

Lymph Node Status	Total No.	%	The Pattern ER+,PR+ &HER-2-ve		The Pattern ER+,PR+ &HER-2+		The Pattern ER-,PR-&HER-2+		The Pattern ER-,PR-&HER-2-ve	
			No.	%	No.	%	No.	%	No.	%
No LN Metastasis	34	26.6	21	29.2	2	22.2	3	21.4	8	24.2
Total LN Metastasis	94	73.4	51	70.8	7	77.8	11	78.6	25	75.8
1 - 3	42	32.8	24	33.3	2	22.2	4	28.6	12	36.4
4 – 9	44	34.4	22	30.6	5	55.6	5	35.7	12	36.4
10≥	8	6.2	5	6.9	0	0	2	14.3	1	3
Total	128	100	72	100	9	100	14	100	33	100

Table (i): The frequency of hormone receptors status (ER and PR) of the breast carcinoma in some studies.

Studies	Year	Region	(%) of ER	(%) of PR
Current Study	**2015**	**Mosul/Iraq**	**63.3**	**63.3**
Mahmoud M M. [8]	2014	Kirkuk /Iraq	50.7	47.8
AL-Bedairy IHM et al. [1]	2014	Baghdad/Iraq	48	46
Mayad IY. [9]	2013	Duhok/Iraq	51.9	55
Ahmed HGA et al. [10]	2011	Yemen	43.8	27
Mudduwa LBK. [11]	2009	Pakistan	32.7	25.3
Hedayati MR. [12]	2008	Iran	46.6	43.8
Ayadi L et al. [13]	2008	Tunisia	59.4	52.3
Rashed MM et al. [114]	2007	Egypt	40.9	31.4
Ahmed HG et al. [15]	2007	Sudan	90	57.5
Sughayer MA et al. [16]	2006	Jordan	50.8	57.5
Yarney J et al. [17]	2005	Ghana	43.2	17.6
Colomer R et al. [18]	2005	Srilanka	35.1	40
Al - Alwan NAS. [19]	1998	Baghdad	61.9	52

The mean age of patients with positive ER&PR expression was 57.4 years as opposed to 45.7 years among patients lacking hormonal expression with P value 0.002. Similarly, 53.1% of patients 50 years or older were ER&PR positive as opposed to 31.9% of patients less than 50 years old; this difference was statistically significant with P value 0.008. These findings are in agreement with other reports in the literature, which show asignificant association between hormone receptors expression in breast carcinoma patients and age at the time of diagnosis[7,27, 37]. In contrast, to study done in Baghdad by AL-Bedairy et al. reported that there is no significant association between them[1].

In the current study, in a group of women aged less than 50 years, HER-2 over expression was found in 56.5% of cases, while in women aged 50 years and older, HER-2 over expression was detected in 43.5% of cases. It should be pointed out that higher rates of HER-2 over expression in young patients have been documented in previous studies[1,7,8, 13,27,30,33]. It is known from the literature that in a group of premenopausal women with breast cancer, worse prognosis of the disease is associated with over expression of HER-2 because it points to a more aggressive form of cancer [1,7,8,13,27,30].

Table (ii): The frequency of HER-2/neu over expression of breast carcinoma in some studies.

Studies	Year	Region	(%) of HER-2/neu
Current Study	**2015**	**Mosul/Iraq**	**18**
Mahmoud M M. [8]	2014	Kirkuk /Iraq	41.3
AL-Bedairy IHM et al. [1]	2014	Baghdad/Iraq	14
Arafah M. [28]	2010	Sudia Arabia	35.4
Rana SA et al [29]	2010	Iraq	41.3
Mudduwa LBK. [11]	2009	Pakistan	24.7
Shuaib HS et at. [30]	2008	Mosul/Iraq	37
Ayadi L et al. [13]	2008	Tunisia	25
Hedayati MR. [12]	2008	Iran	52
Rashed MM et al. [14]	2007	Egypt	40
Sughayer MA et al. [16]	2006	Jordan	17.5

Table (iii): The percentages of the pattern of expression of the triple markers in carcinoma of the breast in different studies.

Studies	Region	Year	No. of cases	The pattern of ER+,PR+&HER -2-ve	The pattern of ER+,PR+&HER -2+	The pattern of ER-,PR-&HER-2+	The pattern of ER-,PR-&HER-2-ve
Current Study	**Mosul/Iraq**	**2015**	**128**	**56.3%**	**7%**	**10.9%**	**25.8%**
AL-Bedairy IHM et al. [1]	Baghdad/Iraq	2014	50	54%	4%	10%	32%
Glu¨ck S. et al [2]	USA	2013	437	6%	10%	47%	37%
Shushan SJ. [3]	Bangalore	2013	60	50%	21.7%	23.3%	5%
Yanagawa M. et al [4]	Japan	2012	363	30.6%	26.2%	11.3%	12.9%
Calderón-Garcidueñas et al [5]	México	2012	506	56.3%	8%	16.6%	19.1%
Recăreanu F. et al [6]	Craiova	2011	75	70%	8.3%	7%	14.7%
Onitilo AA. et al [7]	Marshfield	2009	1134	68.9%	10.2%	7.5%	13.4%

Table (iv): The range and the mean of age distribution of the patients with carcinoma of the breast in different studies.

Studies	Year	Region	Range of years	Mean Year
Current Study	**2015**	**Mosul/Iraq**	**20 - 70**	**49.1**
Mahmoud M M. [8]	2014	Kirkuk /Iraq	16 - 75	47
AL-Bedairy IHM et al. [1]	2014	Baghdad/Iraq	21 - 75	48.2
Shushan SJ. [3]	2013	Bangalore	33 - 80	51.4
Cubukcu E. et al [33]	2013	Turkey	20 - 82	51.9
Recăreanu F. et al [6]	2011	Craiova.	20-80	64
Ambroise M. et al[35]	2011	India	24 to 99	53.8
Onitilo AA.et al [7]	2009	Marshfield	28 - 96	62.7
Lobna A. et al[1]	2008	Tunisia	22 - 89	51.5
Neven B. et al[37]	2007	Belgium	35 - 65	57

In this study, the pattern of expression of the triple markers failed to show statistically significant association with the age of patients with carcinoma of breast, with p value 0.28.This result is in agreement with that reported by other studies [1].

Relationship between the pattern of expression of the triple markers and the size of tumor mass in patients with carcinoma of the breast

In the present study most of the tumors were in group T2 ,conflicting observation were reported by others [3,7,8,10,39]. In contrast, in a study from a western country, the tumors are predominantly in group T1[36], this could be due to the early detection programs prevalent in the western countries and absence of efficient national breast cancer screening program in Iraq and high rate of malignant breast tumors in our country with poorly differentiated cells (aneuploid) **[32].**

In the present study, there is negative association between the expression of hormones receptors and tumor size, similar finding was reported by other study**[40].** Our results show a tendency of HER-2 over expression to be more associated with larger tumor size. Tumors with strong HER-2/neu over expression tended to be larger than those lacking HER-2/neu over expression, with mean sizes of 4.9 cm and 4.3 cm,

respectively. Although this difference was not statistically significant. This finding is in agreement with that reported by others [1,8,27,39].

Relationship between the pattern of expression of the triple markers and the histopathological types of carcinoma of the breast

Histopathological typing revealed that IDC (NOS) was the leading tumor type of the sampled group representing 78.9 % of cases, though other pathologic types were also seen, but extremely with lower frequency, as shown in table (1). Which is comparable with the findings of other studies [3,7,8,27,28]. In this study, some limitations should be considered when interpreting the results, because our study was limited by relatively small numbers of non ductal subtypes were resulting in estimates with wide confidence limits.

No statistical significant difference was found between hormone receptors and histological types of breast cancer in the present study, these results are similar with findings observed by other studies[1,8,28,40]. HER-2/neu was expressed in 20.8% of the invasive ductal carcinoma cases compared to only 10% of our lobular carcinoma cases. This pattern of low HER-2/neu over expression in lobular carcinoma is in agreement with data reported in the literature [8,12,13,28,33].

Relationship between the pattern of expression of the triple markers and the grade of carcinoma of the breast

In the present study, the majority of cases (99.2%) were in grade II and III cancer, while Grade I tumor constitutes only (0.8%), different results obtained by other studies [3,7,8,10,28,41,42], this explains the occurrence of early ages with advance grade during diagnosis. A negative correlation was found between hormonal receptor and histological grade. Therefore, there is a uniform loss of ER content as the tumor becomes more anaplastic indicating that the hormone receptors status could represent one aspect of the tumor cell differentiation, as the grade increase (cell differentiation decrease) and hormone expression decrease. Similar results have been reported by others [38,39].

Correlation of HER-2/neu over expression and tumor grade was also studied by Rakhal EA et al with[42] a sample size of 1,210 cases, showed that HER-2/neu over expression was associated with higher tumor grade, as observed in 3.9%, 20.4% and 38.9% grade I, II and III tumors respectively, whereas in the current study positivity was shown in 0% of grade I, 43.5% of grade II and 56.5% of grade III tumors, similar results were observed by others[27,30,33]. This might reflect the fact that grade I and II in general carry a better prognosis and often associated with ER and PR expression, whereas HER-2/neu over expression and amplification generally associated with aggressive tumors of grade III with a poor prognosis. In contrast, other studies failed to detect this association [1].

Relationship between the pattern of expression of the triple markers and the lymph node status in patients with carcinoma of the breast

In developed countries, majority of the patients the lymph nodes were not involved [36]. Asian studies have documented a greater percentage of breast cancer with lymph nodal metastasis compared to the Western figures [3,7,8, 39]. In the present study, lymph node metastasis by the tumor was noted in 73.4%, which was lesser than that reported in another study in Iraq 81.6% [41].

In the present study the ER and PR receptors status have inverse association with lymph node involvement. Similar findings were reported by others [13,27-29]. Other studies have shown a direct relationship between lymph node metastases and HER-2/neu over expression[30,39]. Our data reveal that 56% of HER-2/neu over expressing tumors had lymph node metastases, as opposed to 42% of HER-2/neu negative cases, although this difference was not statistically significant. This result is in agreement with data reported in the literatures[27,43]. We believe that the low number of cases with known nodal status is responsible for the lack of significant correlation in this study; therefore, future studies with larger numbers of patients are needed to confirm the association of HER-2/neu over expression with nodal metastases.

CONCLUSIONS

HER-2/neu over expression was found in 18% of breast carcinoma in Nineveh Province (north of Iraq). This over expression is associated with some known bad prognostic factors, such as young age at presentation, large tumor size, high histological grade, lymph node metastases and lack of ER and PR expression. In contrast, each of hormonal receptors (ER&PR) were expressed in 63.3% of the cases, they were seen in older patients, and were associated with small tumor size and low histological grade. These data was considered hormone receptor positive and they were likely responded to hormonal therapy. The triple immunonegative pattern was observed in 25.8% of the cases indicating bad prognosis and are not amenable to both hormone therapy and chemotherapy.

The pattern of expression of the triple markers was statistically significant correlate with histopathological type, grade and lymph node status, while they failed to show significant association with the age of patients and size of the tumor mass

Hence, the immunohistochemical assessment of the pattern of expression of the triple markers should be incorporated as a routine investigation. This with histopathological grading and staging will guide the clinicians to make a correct choice of treatment protocols and assessing the prognosis of the patients.

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