Breast Cancer in Kirkuk City, Hormone Receptors Status (Estrogen and Progesterone) and Her-2/Neu and Their Correlation with Other Pathologic Prognostic Variables

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Abstract

Background: Breast cancer shows different clinic- pathological features according to geographic and ethnic groups variation. The ER, PR and Her-2/neu positive status is an indication that the patient is good candidate for hormonal and immunological therapy.

Aim of the study: To measure the frequency of estrogen receptor (ER), progesterone receptor (PR), and Her 2/neu positivity and to study their association with other variables in a breast cancer in Kirkuk city.

Material and method: A prospective study of 138 female breast cancers in Kirkuk city from October 2012 to December 2013.

Results: The age of the patients ranged from16 to 75 years, with a mean of 47 years, most common age group involved was 41-50 years, majority of our patients were less than 50 years, tumor grade III, tumor size between 2-5cm. ER, PR and Her-2/neu expression was seen in 50.7, 47.8 and 41.3% respectively, a characteristic association also found between hormone receptor and Her-2/neu expression and various clinic-pathological parameters.

Conclusion: The hormonal receptor expression appear to be lower and Her-2/neu expression appear to be higher in our population compared to the West and a significant reverse association was found between hormone receptor and Her-2/neu.

Key words: Breast cancer, hormone receptors and Her-2/neu.

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Introduction

Breast cancer is the most common malignancy of females all over the world and the second leading cause of death due to malignancy among females. There is a variation in its incidence among multicultural populations, which suggests that etiological factors differ in their biological expression and impact on the disease outcome [1]. In Iraq according to Ministry of health/Iraqi cancer registry 2006, breast cancer was most frequent cancer among women also it was the commonest in Kirkuk city. It forms 15-32% of total cancer patients with sharp increase in incidence of this tumor in young age group. The average age of patients with breast carcinoma in Iraqi females is 45 years [2].The exact causes of breast cancer are largely unknown, but the environmental & genetic factors are involved. Specific mutations in genes called Her2, BRAC 1, BRCA 2 and P53 have been linked to breast cancer [3]. The prognosis and management of breast cancer is influenced by classic variables as the histological type, grade and stage, status of ER, PR and more recently Her-2, BRAC 1 status, early diagnosis, presence and absence of invasion of axillary and internal mammary lymph node, tumor necrosis, skin and nipple invasion, lymphatic

and vascular invasion, cell proliferation marker (ki-67), type of therapy, local recurrence and gene expression proliferation[4].To date, the majority of risk factors for breast cancer are related to reproductive hormones. Epidemiologic data, animal models, and in vitro studies have shown that reproductive hormones, particularly estrogen, play a critical role in breast cancer etiology via steroid receptors, mainly estrogen receptors (ER) and progesterone receptors (PR)[5]. Estrogen receptor and progesterone receptor expressions are the most important and useful predictive factors currently available. ER and PR are intracellular steroid hormone receptors which have received substantial attention since 1986. Measurable amounts of ER and PR are found in about 50-85% of patients with breast cancer. The frequency of positivity and the level of ER and PR increase with age, reaching their highest levels in postmenopausal women[6]. The expression of ER is seen in 70-80% of cases and PR in 60-70% of cases of invasive ductal carcinoma [7].For the last 25 years advances in the molecular biology have highlighted numerous tumor associated markers in addition to steroid receptors, the most promising of these new was the HER2/neu[8], a protein overexpressed in 18 to 20% of female breast cancers, having both predictive and prognostic values[9]. HER-2/neu importance as a predictive factor is in providing information on the likelihood of response to therapy with anti HER-2 agents, which include the monoclonal antibody (MoAb) trastuzumab (Herceptin)[10] and the small molecule tyrosine kinase inhibitor, lapatinib (Tykerb)[11]. In addition to its value as a marker to predict benefit from certain therapies, overexpression of HER-2 is also associated with higher grade tumors that are more likely to metastasize, therapy protending a worse prognosis in the absence of therapy[12]. Immunohistochemistry (IHC) is the most commonly used method for evaluation for ER, PR & HER-2/neu protein expression on formalin-fixed paraffin-embedded sample of breast cancers [13]. Tumor heterogeneity of breast cancer is one of biggest challenges to be faced, considering that tumors with the same histological types, stages, and degree of differentiation may have different outcomes in relation to prognostic factors and response to implemented treatments [14].

Aim of the study

To measure the frequency of estrogen receptor (ER), progesterone receptor (PR), and Her- 2/neu positivity and to study their association with other variables in a breast cancer in Kirkuk city.

Material and Method

A prospective study was carried out on a total number of 138 breast cancer cases were diagnosed in a period from October 2012 to December 2013. Cases were collected from Azadi teaching hospital, Kirkuk general hospital and from a private laboratory. All of them had modified mastectomy operation (mastectomies with axillary lymph node dissection), either preceded by lumpectomy or one stage mastectomy proceeded by fine needle aspiration cytology (FNAC). The specimens were fixed in 10% fomaline and preceded routinely then embedded in paraffin blocks and four microns-thick sections were obtained and stained with routine hematoxyline and eosin stain. Slides for immunohistochemistry were stained for ER, PR, and HER2/neu using antibodies, buffers and linking systems purchased from DAKO™ (Dako, Denmark). ER used clone SP1, PR clone PgR 636, and HER2/neu polyclonal rabbit anti-human c-erbB-2. Immunohistochemistry was performed according to manufacture instructions. The linking/amplification system consisted on EnVision™ system polymer-enzyme conjugate (Dako, Denmark). The cases were

classified according to WHO classification. All available data regarding demographic and prognostic histopathological features were collected and analyzed. Histologic features were; histopathology subtype, tumor size, number of lymph nodes, tumor grade, ER and PR status and HER2/neu oncogene overexpression. Nottingham Modification of Bloom-Richardson Grading Scheme was used for grading. Expression of ER and PR were assessed after the entire immunostained slides had been evaluated by light microscopy, and results were determined by assigning a proportion score and the intensity score according to Allred scoring system [15]. HER2/neu was assessed according to HercepTest™ Guidelines for scoring of HER2/neu[16]. Data was analyzed by using Statistical Package for Social Sciences (SPSS) version 19 for windows.

Results

Total number of 138 cases of breast cancer has been included in the present study. The age distribution of the patients ranged from (16 to 75) years. The overall mean and median ages were 47 years and 48 years respectively. Most common age group involved was (41–50) years (39%). Eighty cases were below the age of fifty years, while remaining 58 cases were fifty one years old or more as shown in table (3). Thirty nine point one percent were in postmenopausal period. Malignancy was in the left breast in (54.3%) of patients and in (42%) of cases was found in the right, while just 5 patients (3.7%) had bilateral tumor. Tumors were located in upper lateral quadrant in (48%) of cases, whereas other quadrants & locations had an involvement of lesser extent, as shown in table (4). Regarding the pathological features of the patients, the leading tumor type was invasive ductal carcinoma not otherwise specified (NOS) (93.5%), figure (1), though other pathologic groups were also seen, but with extremely lower frequencies figure (2). Tumors were mainly in grade III (46.4%). T2 (tumor measuring 2-5cm) tumor was found in (52.7%) of cases, while T1(tumor measuring <2cm) and T3(tumor measuring >5cm) constitute lower percentage (12.0% and 35.3% respectively), as shown in table (4). Tables (5) and (6) give the estrogen receptor (ER), progesterone receptor (PR) and Her-2/neu status by tumor grade, size, lymph node involvement and histological type.

The percentage of ER positive breast cancer is (50.7%), followed by PR positive (47.8%), figures (3) and (4) and Her-2/neu over-expression is (41.3%), figures (5) and (6).

There is a significant negative correlation between ER, PR and grade of tumor (P value <0.001), tumor size (P value <0.001) and the number of lymph node involved (P value <0.001).

Inversely there is a significant positive relationship between Her-2/neu and grade of the tumor, tumor size and the number of lymph node involved (P value <0.001) as shown in (table 5), however no such statistically significant correlation found with histological type (P value >0.05).

It has been found that there is an inverse correlation between ER and HER2 which is statistically significant (p value <0.001) and the vast majority of ER positive tumors were lacking Her-2/neu (93%). Similarly the vast majority of PR positive tumors (91%) were Her-2/neu negative; as shown in table (6).

Table (3): Age distribution of the presented breast cancer patients**.**

Age groups(years)	Number (%)
20≤	2 (1%)
21-30	5 (4%)
31-40	19 (14%)
41-50	54 (39%)
51-60	32 (23%)
61-70	18 (13%)
>70	8 (6%)

Table(4): Clinicopathological characteristics of patients with breast cancer**.**

Factor	Value No. (%)
No.	138 (100%)
Age at diagnosis (years)	
50≤	80 (58%)
>50	58 (42%)
Age range (year)	16-75
Number of positive lymph node	
0	21 (15.2%)
1-3	52 (37.7%)
>3	65 (47.1%)
Tumor size (cm)	
T1 (< 2)	17 (12.0%)
T2 (2-5)	73 (52.7%)
T3 (>5)	48 (35.3%)
Histological grade	
I	24 (17.4%)
II	50 (36.2%)
III	64 (46.4%)
Tumor laterality	
Left	75 (54.3%)
Right	58 (42%)
Bilateral	5 (3.7)
Histological type	
Ductal	129 (93.5%)
- Invasive ductal carcinoma (NOS)	121
-Medullary	3
-Papillary	3
-Mucinous	2
Lobular	9 (6.5%)
Menopausal status	
Premenopausal	84 (60.9%)
Postmenopausal	54 (39.1%)

Table (5): ER, PR and Her-2/neu status by tumor grade, lymph node involvement tumor size and histological type.

Factor		No.of cases n=138	ER		PR		Her-2/neu	
			Positive No.(%)	Negative No.(%)	Positive No.(%)	Negative No.(%)	Positive No.(%)	Negative No.(%)
Grade	I	25	24(96)	1(4)	23(92)	2(8)	0(0)	25(100)
	II	47	30(63.8)	17(36.2)	31(66)	16(34)	10(21.3)	37(78.7)
	III	66	16(24.2)	50(75.8)	12(18.2)	54(81.8)	47(71.2)	19(28.8)
Total		**138**	**70**	**68**	**66**	**72**	**57**	**80**
p-value < 0.001								
Lymph node involvement	0	21	20(95.2)	1(4.8)	19(90.4)	2(9.6)	0(0)	21(100)
	1-3	52	35(67.4)	17(32.6)	33(63.5)	19(36.5)	13(25)	38(75)
	>3	65	15(23)	50(67)	14(21.5)	51(78.5)	44(67.7)	21(32.3)
Total		**138**	**70**	**68**	**66**	**72**	**57**	**80**
p-value < 0.001								
Tumor size (cm)	<2	17	14(82.4)	3 (17.6)	12(70.6)	5(29.4)	1(5.8)	16(94.2)
	2-5	73	52(71.2)	21(28.8)	50(68.5)	23(31.5)	23(31.5)	50(68.5)
	>5	48	6(12.5)	42(87.5)	4(8.3)	44(91.7)	33(68.8)	15(31.2)
Total		**138**	**70**	**68**	**66**	**72**	**57**	**80**
p-value < 0.001								
Histological type	IDC	129	68(52.7	61(47.3)	63(48.8)	66(51.2)	53(41)	76(59)
	ILC	9	2(22.2)	7(77.8)	3(33.3)	6(66.7)	4(44.4)	5(55.6)
Total		**138**	**70**	**68**	**66**	**72**	**57**	**80**
p-value > 0.05								

Table (6): Correlation of Her-2/neu with ER and PR.

Hormone receptor		No. Of Cases	Her-2/neu +ve	Her-2/neu – ve	p-value
ER status	Positive	70	5	65	<0.001
	Negative	48	52	16	
PR status	Positive	66	6	60	<0.001
	Negative	72	51	21	
Total		138	57	80	

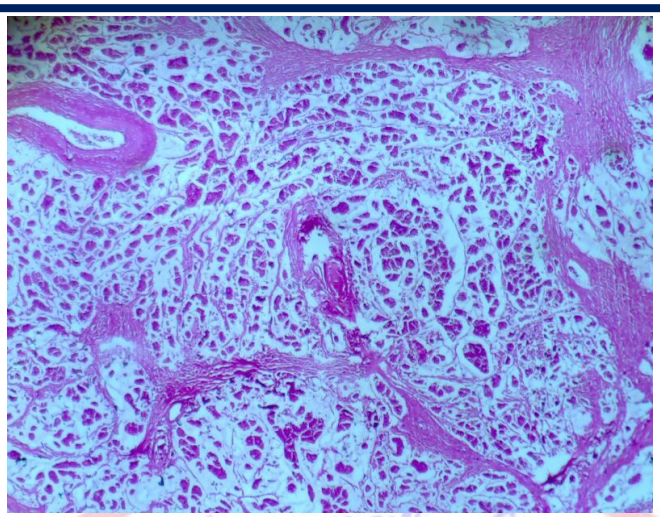

Figure (1): Invasive ductal carcinoma(NOS) (x100H&E).

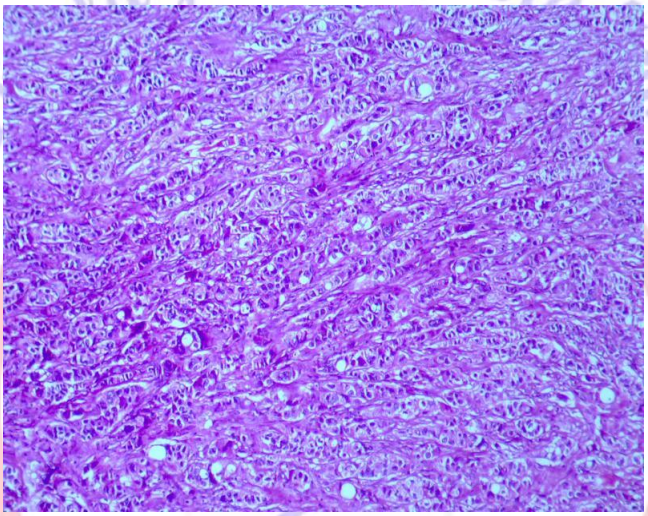

Figure (2): Mucinous carcinoma (x100 H&E).

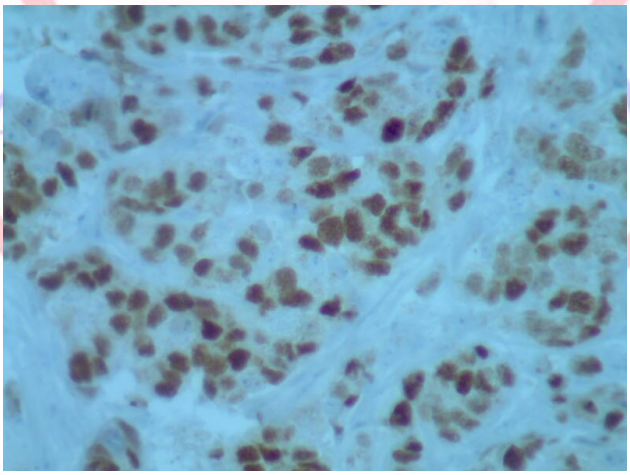

Figure (3): Breast carcinoma with ER positive (Immunohistochemistry x100).

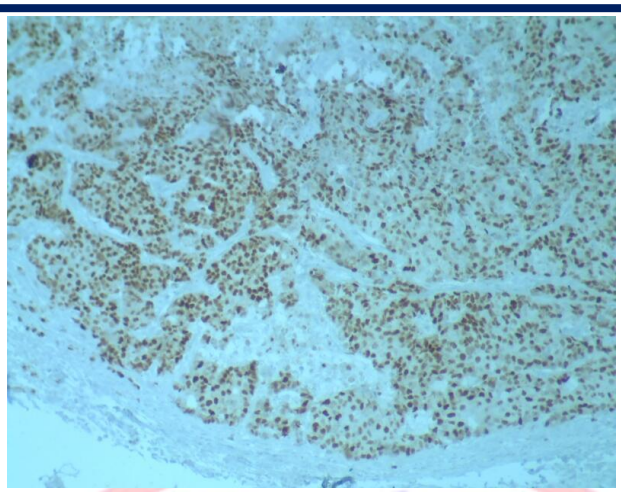

Figure (4): Breast carcinoma with PR positive (Immunohistochemistry x40).

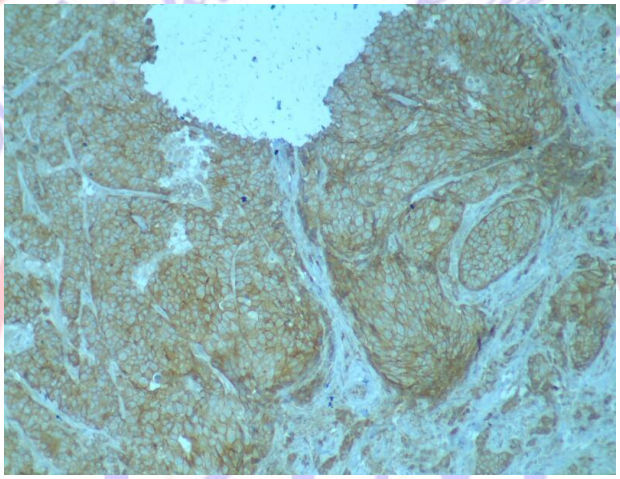

Figure (5): Breast carcinoma with Her-2/neu positive (Immunohistochemistry x100).

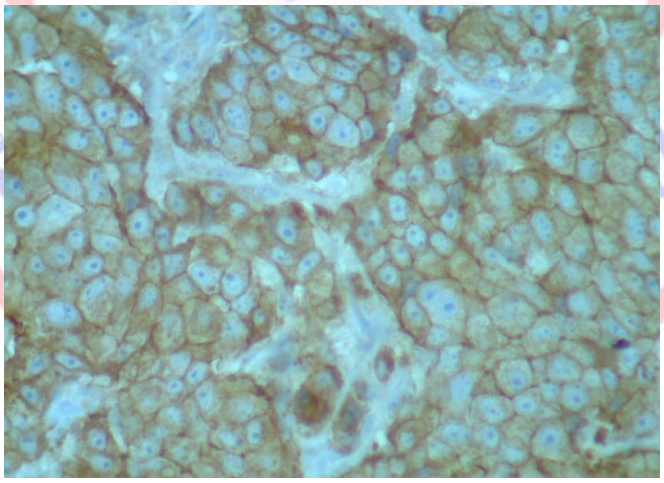

Figure (6): Breast carcinoma with Her-2/neu positive (Immunohistochemistry x40).

Discussion

The incidence of breast cancer is increasing in all industrialized countries. Changes in diet and reproductive patterns and altered exposure to endogenous and exogenous substances with hormonal activity have been suggested as a contributing to this increase. Breast cancer remains the most common cancer among Iraqi women and constitutes a real problem of public health. According to Globocan 2008, the incidence of breast cancer was of 31.1 per 100.000

women in Iraq. This incidence rate was widely lower than the rates observed in most developed countries, such as United States [age-standardised incidence and mortality rate (ASR):76.0], United Kingdom [ASR: 89.1], Canada [ASR: 83.2], Denmark [ASR: 101.1][17]. Nevertheless, the incidence rate reported here remains superior to rates reported from Asia.

The results of the present study revealed that the ages of the breast cancer patients were ranging from 16-75 years, with a mean age of 47 years, with 58% were younger than 50 years. The peak age frequency of 41-50 years was reported in this study, which included 39% of the breast cancer patients. Al-Alwan reported close results in a study carried out in Baghdad with peak age frequency between 40-49 years [18]. Similar peak age frequency recorded by the Iraqi Cancer Registry 2006[2], and in other reports from our country (Al-Janabi[19], Al-Anbari[20], Ahmed NY[21], Yalda MI[22]). The relatively low mean age of breast cancer patients reported in this study (47 years) is close to that reported by other investigators in Baghdad (46.5 years) [19] and Egypt (46 years)[23]. Slightly lower mean ages reported in Bangladesh (45.6 years)[24] and Kingdom of Saudi Arabia[25] while slightly higher mean age (48 years) reported in Tunisia[26]. Higher mean ages reported in Palestinian Arab Kindred and Jewish population (51.5 and 55.9 years respectively) [27].

The median age of onset of the patients (47years) is more than ten years younger than that reported in Europe and USA (61 years) (excluding the African-American). While numerous theories have been proposed to explain this difference, including age at menarche, time of first delivery, parity, socio-demographic factors, body mass index, high fat diet, lack of physical activity, relative marriages which are not uncommon in our community and genetic difference, none are completely satisfactory and more researches are needed in this area to determine the predisposing factors in our patients [28].

In developed countries, majority of the patients the lymph nodes were not involved [29]. Asian studies have documented a greater percentage of breast cancer with lymph nodal metastasis compared to the Western figures [30,31,32]. In the present study the positive lymph node was 84.8%, similar results were seen in another study in Iraq 81.6% [33].

Tumor size in the present study was 2cm and above in 88% of cases. This was comparable to other Asian studies [31,32,34]. In contrast, in a study from a western country, the tumors are predominantly less than 2cm[29], this could be due to the early detection programs prevalent in the western countries and absence of efficient national breast cancer screening program in our country and high rate of malignant breast tumors in Iraq with poorly differentiated cells (aneuploid) [35]. It has been found that the majority of the patients (82.6%) presented with grade II and grade III cancer, while grade I constitutes (17.4%), different results obtained by other studies [20,33], this explains the occurrence of early ages with advance stage during diagnosis.

The hormones receptor status and responsiveness of tumor to hormone therapy is an important parameter in breast cancer management and patient survival. The prevalence of hormones receptor-positive breast cancer in Asian countries has been found to be lower than those in the western world. However, as denoted in this study, the incidence rates of the ER and PR receptors expressions were lower than what were reported in the literature from USA and Australia (65% - 80%). Moreover, these relatively lower ER and PR expressions in the present study (50.7% and 47.8% respectively) were relatively consistent with the lower reported ranges in different Asian

and African countries, as shown in table (7) which indicate that the majority of the breast carcinoma in the study sample would not respond to endocrine therapy. Al-Alwan reported higher occurrence of these receptors in malignant breast tissues [18]..Even higher figures recorded in Western studies [35,36]. Relatively low figures in the present study could be attributed to the low age at presentation; this fact is supported by some observations in several studies [37] also this variation may be related to other 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.

Table (7): Rates of Hormone Receptor Status of Breast Carcinoma in Some ASIAN and African Countries

Country		(%) of ER+	(%) of PR +
Iraq	Dohuk[21]	51.9	55.
	Baghdad[18]	61.9	52
Pakistan[38]		32.7	25.3
Srilanka[39]		35.1	40
Ghana[40]		43.2	17.6
Yemen[41]		43.8	27
Iran[42]		46.6	43.8
Jordan[43]		50.8	57.5
Tunisia[44]		59.4	52.3
Eygpt[37]		40.9	31.4
Sudan[45]		90	77.5

Significant inverse association was found between hormonal receptor and histologic grade. Greater percentage of grade I tumors shows ER, PR positivity when compared to grade III tumors (p value<0.001). 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 shown by other [34].

In the present study the ER and PR receptors status have inverse association with lymph node involvement and tumor size. Similar findings were reported for PR only by Moradi-Marjaneh, et al[46]however, Ayadi, et al[44] didn't find any association between ER and PR expression and clinicopathological factors except negative association with tumor grade. Furthermore, the associations of PR expression with other pathological factors were reported by Mohsin, et al [47]. Only association of ER and PR expression with tumor grade was reported by Adebamowo, et al [48] in addition, to the findings by Lu,et al [49], when they demonstrated a negative association between the expression of hormones receptors and tumor size and tumor grade.

No significant statistical difference (p value >0.05) found between hormone receptors and histological type of breast cancer in the present study, these results agree with findings observed by other studies [50].

The percentage of Her-2/neu overexpression in the current study was 41.3% appear to be comparable to local studies done by Saleem et al in the North of Iraq (37%)[51] and Aziz et al in the Middle of Iraq 41.3%[52] and higher than results of Abbas et al in the South of Iraq 31.5%[53]. Less than 20% or more than 30% of Her-23/neu over expression was reported by many international studies from Middle East and North African countries, as shown in table (8). This variation in Her-2/neu expression may reflect differences in subjective evaluation of Her-2/neu status.

Table (8): Rates of Her-2/neu Overexpression of Breast Carcinoma in Some ASIAN and African Countries.

Studies	(%) of Her-2/neu positivity
Jordan(43)	17.5
Pakistan(38)	24.7
Tunisia(44)	25
Saudia Arabia(54)	35.36
Eygpt(37)	40
Iran(42)	52

An inverse association has been found between Her-2/neu overexpression and the presence of ER and PR; thus the higher the level of Her-2/neu overexpression the lower the corresponding ER and PR positivity. These finding are comparable to other studies [45,46]. This has been explained by hormone-dependent down regulation of Her-2/neu involving a complex molecular interaction. Estrogen and its receptor are required to suppress Her-2/neu [55], this explained the Her-2/neu overexpression in women with low or absent ER expression. But they differ from study in Iran [56] which shows no significant association between hormone receptors and Her-2/neu status.

Correlation of Her-2/neu over-expression and tumor grade was also studied by Rilke et al[57] with 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, 21.3% of grade II and 71.2% of grade III tumors, similar results were observed by others[36] 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 overexpression and amplification generally associated with aggressive tumors of grade III with a poor prognosis.

The present study has shown that there is a strong correlation between Her-2/neu positivity and steroid receptor negativity, as generally described in the literature with a variety of techniques [57]. Her-2/neu negative tumors were more likely to be ER-positive or PR-positive than Her-2/neu positive tumors of invasive breast cancer, Similar results were found by other observer [32].

In conclusion, the patients in the present study were present with low age, higher grade, with lower hormone receptor status and higher Her-2/neu expression, may suggest that the young onset of breast cancer in our patients in Kirkuk city could be attributed to the genetic predisposition which may be modified by the changes in life style factors such as the increased utilization of hormones like contraceptives or fertility treatments that leads to steep rise in the

incidence of breast cancer in this population, therefore, this understudied population can provide important data regarding gene-environment interactions in well-designed studies and our data are closer to that of Estren than Westren world.

Recommendation

1-Implementation of the simple and effective screening programs for early detection is urgent need in our population.

2- Larger studies are required to study the biological behavior of breast cancer in this high risk population, to determine the predisposing factors in our patients.

3-Adjuvant hormonal therapy in our setup should be based on the presence of hormone receptors, as assessed by immunohistochemical study of breast cancer tissue to make national guidelines for treatment; the studies should cover the population from all over the country.

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