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Immunohistochemical Evaluation of Positive Hormone Receptors and HER2 Overexpression in Women with Breast Cancer

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Abstract

Background: Breast cancer has a tremendous heterogeneity in its clinical behavior. The objective of this study is to assess the positive expression of estrogen receptors (ER), progesterone receptors (PR) and HER2 overexpression in relationship to the age of patients, and certain prognostic parameters such as tumor grade, size and lymph node involvement. **Patients and methods:** A cross sectional case study was conducted between June 2011 and June 2014 at the pathology department of Rizgary General Hospital, Erbil, Iraq. 114 Confirmed cases of breast cancer were studied. Immunohistochemistry was used to evaluate the expression of ER, PR and HER2 status. Patients' mean age was 48±5 (Range: 28-83) years; 57% of them were ≤50 years. **Results:** The expression of ER and PR was 58.8% and 49.1% respectively. HER2 overexpression (score +3) was 29.8%. Hormone receptors (ER and PR) correlated significantly with age and grade of the tumor whereas HER2 overexpression correlated significantly with grade and lymph node involvement. An inverse correlation was observed between the distribution of both ER & PR and overexpression of HER2. **Conclusions:** Expression of hormone receptors were evident in about half while HER2 overexpression was observed in less than one third of patients in our population. Different and common correlations were observed between ER, PR and HER2 expression and other patient and tumor characteristics. HER2 overexpression is associated with an aggressive form of breast cancer with high histological grade and negative ER status.

Key words: Immunohistochemistry, Estrogen, Progesterone, HER2, Breast cancer.

Introduction

Breast cancer is the leading female malignancy worldwide accounting for 19% of all female cancers. It is the leading cause of cancer-related death in women. In 2010, nearly 1.6 million women worldwide were diagnosed with breast

cancer, accounting approximately for 14% of the cancer deaths in women and 35% of all cancer cases (1). In Iraq, breast cancer is the commonest type of female malignancy accounting for approximately one third of the registered female cancers according to the Iraqi Cancer Registry (2). In breast cancer, estrogen receptors (ER) and progesterone receptors (PR) are known to be significant predictive and prognostic markers for breast cancer. They act as DNA binding transcription factors and regulate the activity of different genes mediating breast cell proliferation and DNA replication leading to mutation (3). Survival and response to hormonal therapy are most favorable among patients

Table 1. Calculations of the percentage scores and intensity scores used in the Allred score of ER and PR scoring.

Percentage Score		Intensity Score	
Positive cell percentage	Proportion score	Labeling intensity	Intensity score
0	0	No labeling	0
<1	1	Low intensity	1
1-10	2	Moderate intensity	2
10-33	3	High intensity	3
33-66	4		
66-100	5		

Allred score is the sum of the percentage and intensity scores (see text)

Table 2. Clinicopathological characteristics of 114 patients.

Characteristics	Details/Criteria	Number	Percentage
Age (years)	≤ 50	65	57
	> 50	49	43
Tumor grade	I	17	14.9
	II	62	54.4
	III	35	30.7
Tumor size (cm)	<2	32	28.1
	2-5	45	39.5
	>5	37	32.4
Lymph node status	Positive	59	51.8
	Negative	55	48.2
ER expression	Positive	67	58.8
	Negative	47	41.2
PR expression	Positive	56	49.1
	Negative	58	50.2
HER2 expression	Score +3	34	29.8
	Score 0,+1,+2	80	70.2

Table 3. Statistical analysis of the distribution of ER, PR, HER2 in relationship to age, grading, tumor size lymph node involvement.

Characteristics and details +ve		ER status			PR status			HER2 status		
		-ve	All	+ve	-ve	All	+3	0,+1,+2	All	
Age	≤ 50	29	36	65	26	39	65	24	41	65
	> 50	38	11	49	30	19	49	10	39	49
	All	67	47	114	56	58	114	34	80	114
	Statistics	χ^2 = 12.5; df=1; p≤ 0.01			χ^2 = 5; df=1; p≤0.05			χ^2= 3.6; df=1; NS		
Grades	I	11	6	17	9	8	17	9	8	17
	II	43	19	62	39	23	62	13	49	62
	III	13	22	35	8	27	35	12	23	35
	All	67	47	114	56	58	114	34	80	114
	Statistics	χ^2 = 9.91; df=2; p≤ 0.01			χ^2 =9.5; df=2; p≤0.01			χ^2 = 7.1; df= 2; p≤ 0.05		
Tumor size	< 2 cm	18	14	32	18	14	32	8	24	32
	2-5 cm	25	20	45	20	25	45	16	29	45
	> 5 cm	24	13	37	18	19	37	10	27	37
	All	67	47	114	56	58	114	34	80	114
	Statistics	χ^2 = 0.81; df=2; NS			χ^2=1.1; df=2; NS			χ^2 =1.04; df=2; NS		
Lymph node	Positive	37	22	59	30	29	59	24	35	59
	Negative	30	25	55	26	29	55	10	45	55
	All	67	47	114	56	58	114	34	80	114
	Statistics	χ^2 = 0.6; df=1 NS			χ^2 =0.12; df=1 NS			χ^2=6.75; df=1 p≤0.01		

Table 4. Statistical analysis of association between ER, PR and HER2 scoring

HER2 status	ER status			PR status		
	Positive	Negative	All	Positive	Negative	All
+3	13	21	34	11	23	34
0, +1, +2	54	26	80	45	35	80
All	67	47	114	56	58	114
Statistics	χ^2 = 8.49; df=1; p≤ 0.01			χ^2 = 5.44; df =1; p≤0.05		

with hormone sensitive cancers (3). Recently, testing for HER2 has been included in the routine patient's work-up in recognition of its value as a prognostic marker and perhaps particularly in predicting response to Trastuzumab therapy (4). The advances in the production of monoclonal antibodies and in antigen retrieval methods have greatly improved the detection of ER and PR in the sections from formalin-fixed paraffin embedded tissues (5).

The aim of this study was to initiate a database about the frequency and distribution of both hormone receptors (ER, PR) and HER2 in the local populations of Erbil and to evaluate their association with age and other factors.

Materials and Methods

Patients and Specimens

This cross-sectional study was carried out at the Pathology Department, Rizgary General Hospital during the period of June 2011- June 2014. Patients' age ranged from 28 to 83 years with a mean age of 48±5 years. Most of the patients (65, 57%) were 50 years or younger. One hundred fourteen (114) cases of breast carcinoma histologically confirmed were studied (6). There were 97(85.1%) ductal carcinoma, 11(9.7%) lobular carcinoma and 6(5.2%) mixed carcinoma. These confirmed cases were graded using the Scarff- Bloom-Richardson grading system (7) and staged according to the American Joint Committee on Cancer Staging (8). This study was approved by the Ethics Committee of Hawler Medical University, Erbil, Iraq.

Immunohistochemical studies

Staining of the HER2 protein, ERs and PRs were performed for all specimens as previously described (9). A brief description is given here. 4 micrometer sections, attached on salinized slides were de-waxed in Xylene, rehydrated in graded ethanol and covered with 10 mM Citrate buffer (pH 6.0). They were then incubated for 30 minutes with primary monoclonal antibodies against HER2 (DAKO, clone 124,1:100), ER (DAKO, clone 1D5,1/25) and PR (DAKO, clone PgR636,1/50), followed by incubation with biotin-labeled secondary antibodies. The streptavidin-peroxidase complex was visualized using di-aminobenzidine (DAB kit, K3467, Dakocytomation, Denmark) as a chromogenic substrate. Scoring of ER and PR was done according to Allred scoring system. This system takes into consideration both the percentage of labeled cells and the medium intensity of the nuclear labeling. The Allred score is the sum of the percentage score (percentage of labeled cells) and the intensity score (labeling intensity). HER2 was scored on a scale from 0 to 3 according to the Dako criteria. The staining was scored as negative (0) when no membrane staining was observed, or when membrane 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 and complete membrane staining with weak to moderate cytoplasmic reactivity was seen in more than 30% of the tumor cells. In the final analysis, only score (+3) were considered as HER2 overexpression cases. Total score = proportion score + intensity score. Tumors with Allred score ≤ 2 were considered negative and tumors with score > 2 were considered positive for ER and PR.

Statistical analysis

Data are expressed as mean ± standard deviation or as percentage (ranges). Chi-square (χ^2) test was used to explore the differences between groups and $p \leq 0.05$ was considered statistically significant.

Results

More cases were of grade II (54.4%) followed by grade III (30.7%) and grade I (14.9%). The ER positivity, PR positivity and HER2 over-expression were evident in 58.8%, 49.15% and 29.8% of cases respectively. The clinicopathological characteristics of the 114 patients and their tumors are detailed in table 2. Figure 1 illustrates the various classifications.

The distribution of ER, PR and HER2 in relation to different parameters, namely age, grades, tumor size and lymph node involvement are shown in table 3. ER & PR were distributed in a highly significant ($p \leq 0.01$) and significant ($p \leq 0.05$) manner in different ages respectively while the distribution of HER2 was not significant (Table 3). Regarding the factors related to the pathogenicity and aggressiveness of the tumor (grade, tumor size and lymph node involvement), the difference of the distribution of ER & PR in different grades was highly significant ($p \leq 0.01$) for both but the difference was not significant regarding tumor size and lymph node involvement. In the same manner, the difference in the expression of HER2 in different grades was significant ($p \leq 0.05$), highly significant ($p \leq 0.01$) with lymph node involvement and not significant regarding tumor size (Table 3).

The overexpression of HER2 in ER +ve tumors (38.3%) was less than that in ER- negative tumor (61.7%) with a highly significant differences ($p \leq 0.01$) (Table 4). Over-expression of HER2 was also inversely related to PR status, as this overexpression in PR +ve (32.3%) was significantly lower than that in PR–ve tumors (67.7%) (Table 4).

Discussion

Determining an individual prognosis of a breast cancer's

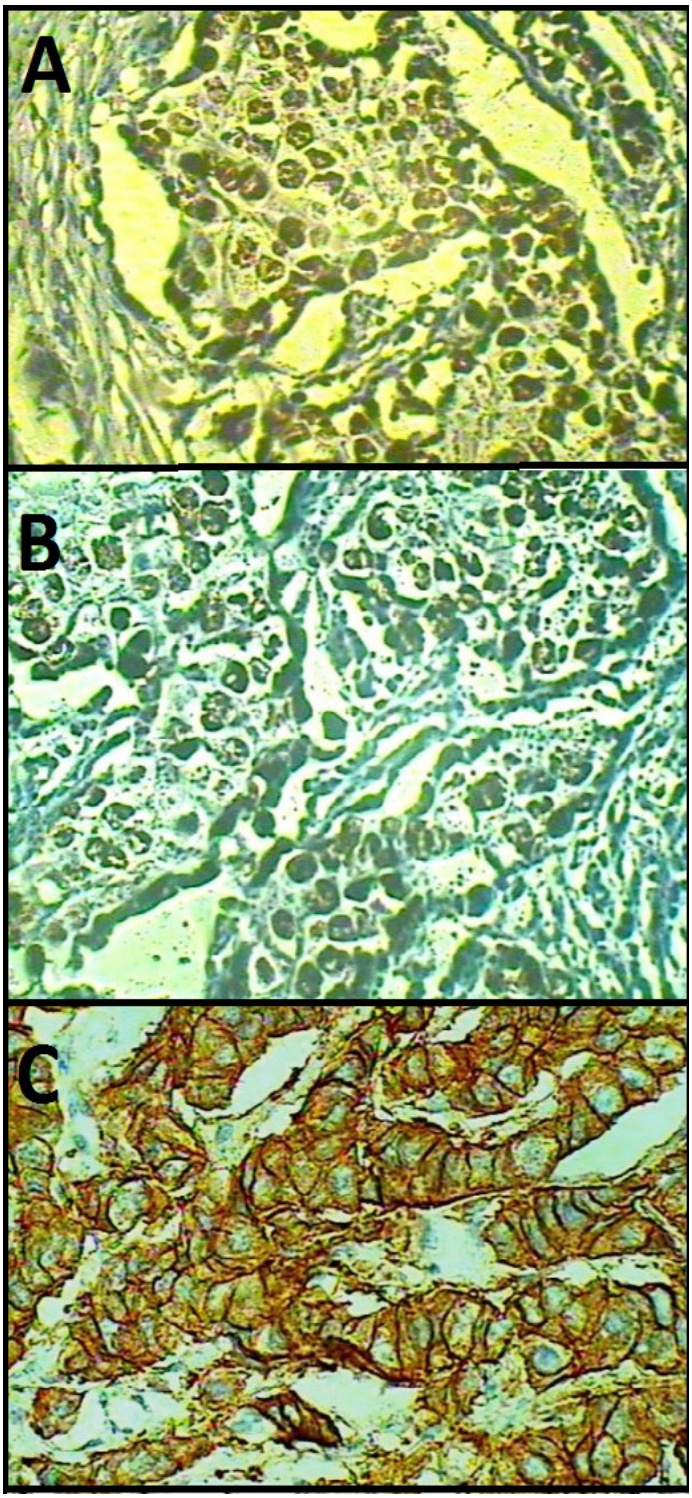

Figure 1. A. ER+ve tumor cells in breast cancer (granular brownish discoloration of nucleus), B. PR+ve tumor cells in breast cancer, and C. HER2 neuimmunostaining: strongly positive (3+) pattern showing intense membranous staining in more than 10% of the tumor cells. x400

patient at time of diagnosis requires a detailed examination of as many clinical, pathological and anatomical parameters as possible (1). Prognostic factors in breast cancer are indicators that reflect the individual characteristics of the tumor and the outcome of the patient. Analysis and evaluation of these factors play a fundamental role in selection of the most effective cancer specific therapy with the least unnecessary toxic effects produced by inadequate treatment regimens (2). Our reported work relied on immunohistochemistry being the most commonly used method of testing for ER, PR and HER2 status. The combined expression of ER, PR, HER2 and other markers has thus become most informative in molecular classification of breast tumors and their clinical assessment for treatment and outcome (4,5).

Tables 3 and 4 demonstrate that the differences in the distribution of ER in both age groups and different grades were highly significant while the differences regarding PR are significant and highly significant in both factors respectively. The ER expression rates noted in this study are in agreement with results of other regional studies from Baghdad, Jordan, Saudi Arabia, Tunisia and Egypt ranging from 53 to 65% (4,5,10-13). This is however different from those reported in Austrian women (80.6%), American white non-Hispanic women (63.9 %) and Nigerian women (24%) (14-16). It is established that the hormonal status differs amongst different ethnic groups (14). Analysis of 6 studies found that African-American women had higher ER-ve rates than Hispanics and White Americans (15). Also, higher ER-ve/PR-ve rates were reported in Asian Fillipino breast cancer patients compared to Asian American women (16). In addition, ER status of Malay, Chinese and Indian women with breast cancer living in Singapore and Malaysia was reportedly different amongst these three ethnic groups (17). The variation of hormonal status among breast cancer patients with different races may be attributed to genetic disparities and socioeconomic factors such as life style, nutritional status and environmental exposure (14-16). Unfavorable cancer types such as HR negative, HER2 overexpression or high-grade tumors are seen more frequently in certain races (17). ER-ve tumors are more frequent in some hereditary breast cancers that bear BRCA1 mutation (14,15). Regarding socioeconomic factors, it had been reported that low socioeconomic status may be related to HR- e breast cancer (12,13). On the other hand, disparity of these results was attributed to pre-analytic variables which can lead to incorrect results including the use of fixative other than 10% neutral buffered formalin (unless

that fixative has been validated by the laboratory before offering the assay) or biopsies fixed for intervals shorter than 6 hours or longer than 72 hours (18). Navani and Bhaduri suggested that ER seems to be more vulnerable to pre-analytic variables as they showed higher numbers of ER-ve/PR+ve cases most of which subsequently turned out to be ER+ve/PR+ve when repeated with a different set of antibodies using automated immunohistochemistry (19). In the present study, younger patients were more susceptible to have breast cancer without ER expression than older patients. This is consistent with other studies from, Saudi Arabia, Tunisia, Jordan Iran, and Cuba (10,11,20-22). Yip et al proposed that age is associated with ER positivity as the postmenopausal women displayed higher rates of ER positive tumors (22), whereas Yalda (4) suggested that either the low ER expression observed in premenopausal women might represent a true lack of the receptor protein or alternatively the tumors in elderly women tend to be better differentiated and hence having higher receptor contents and these could be attributed to the genetic predisposition. The present study showed that half of the patients harbored PR +ve receptors in their cancer and a significant association between age of the patients and their tumor expression of PR was noted. These findings are similar to those in several previous studies (4,5,10-12) but at contrast with others (13). Most of our patients were of grade II and hormone receptors were distributed with highly significant differences between the grades. As the tumor grade increases, an increase in loss of ER and PR expression was noted. This may indicate a uniform loss of both receptors as the tumor become anaplastic indicating that hormone receptors status could represent one aspect of tumor cell differentiation (2,11). Yip et al proposed that despite being associated with a higher grade, ER status is an independent factor for survival, with ER positive cancers having better overall survival (22). PR exist in two variants (PRa & PRb). They come from the same gene but are regulated by two different estrogen regulated promoters. In breast tissue, PRa & PRb are similarly expressed while in atypical hyperplasia, non-invasive and invasive breast cancers are both heterogeneously expressed in adjacent cells and PRa is much more often expressed in adjacent cells than PRb in noninvasive and invasive cancers. This indicates that loss of control of PR expression is an early event in breast carcinogenesis (11,13). PR lost its independent predictive value in the 1990s; but later studies associated the loss of PR expression with an increase in growth factor signals and aggressiveness of the tumor. PR has now regained importance and redefined as a predictive marker of ER activity and also as a fundamental marker for indicating hormone therapy in breast cancer patients. When accurately measured, PR status is an independent predictive factor for benefit from adjuvant endocrine therapy with Tamoxifen. On the other hand, the interrelationship between the steroid hormone receptors and cytokines, including TNFα, has been demonstrated in several studies. Increased endogenous TNFα may promote tumor invasion via down-regulating the PR expression in breast cancer (19-21). In addition, TNFα was more abundant in PR negative cancers than in positive ones (22). Moreover, TNFα has an important role in regulating estrogen synthesis in malignant breast tissues. ER may also inhibit TNFα activation via repressing the TNF-responsive element (TNF-RE) and TNF promoter (23,24). In the present study, no significant difference in distribution of ER & PR in relationship to tumor size and lymph node involvement factors was detected (Table 4).

Hormone receptors content were not shown to have noticeable relation with tumor grade, tumor size and histological type while the ER & PR receptors status had a positive association with lymph node involvement (23). These findings were reported previously for PR only (24). Ayadi et al could not find any association between ER and PR expression and Clinicopathological factors except for a negative association with tumor grades (11). An association of PR expression with other pathological factors was reported by some workers (25), while only association of ER &PR expression with tumor grade was reported by others (26). In the present study, the percentage of HER2 overexpression is similar to that reported in regional studies noted above (11-13) but at variance from findings from Iran that showed a higher percentage of HER2 expression at 46% (20). This variation in expression may reflect differences in the evaluation of HER2 status. No significant association has been found between HER2 overexpression and age in our study; at variance from several other studies that showed a significant inverse correlation between age and HER2 overexpression (2,12,14,20). It also differed from the study from Saudi Arabia (16) which showed significantly higher HER2 overexpression among postmenopausal women. However, our results are in agreement data from Egypt that also indicated no significant association between HER2 over-expression and age of the patients (13). These differences could be also due to the inherently subjective nature of the HER2 scoring. Though tumor size is predictively an important prognostic factor for patients in cases both with or without positive axillary nodal metastasis (27), our data didn't detect any significant

difference in the distribution of score +3 and other scores in different tumor sizes. HER2 overexpression was varied with grades and lymph node involvement in a significant and highly significant manner. Poorly differentiated invasive ductal carcinoma has been shown previously to have higher frequency of HER2 positive tumors than moderately and well differentiated tumors (28). The prognostic importance of HER2 expression has also been analyzed in the context of patient subgroups with or without lymph nodes involvement. Most studies that examined the prognostic role of HER2 in patients with positive lymph nodes have shown that HER2 expression was associated with a worse outcome in either univariate or multivariate analysis (11,13,14,16). Despite the great variation in levels of HER2 positivity, several groups reported a negative relationship between HER2 status and steroid receptors levels consistently (21,24,27) but not invariably (20).

Results of the present study confirm the inverse correlation between HER2 and ER and to some extent between HER2 and PR. Numerous studies indicated that, presence of mutually repressive feedback signaling loop between ER and HER2 resulting in inverse correlation and probably reflecting the interrelationship of endocrine and paracrine signals important in normal gland development as well as cancer (29). It could be suggested that the dominant effect of ER and HER2 cross talk is alteration of ER gene transcription and the net result could be altered responsiveness to endocrine manipulation.

In conclusion, the present study demonstrates that expression of HR's were evident in about half of the cases and HER2 overexpression in less than one third of cases. HR expression were related significantly to patient's age and grade of the tumor while HER2 overexpression was related significantly to grade and lymph node involvement. There was a significant inverse relationship between the distribution of HR's expression and the overexpression of HER2. Apparently, HER2 overexpression presents an aggressive form of breast cancer with high histological grade and negative ER. We suggest that HER2 status in breast cancer is vital as it provides a valuable prognostic, predictive and therapeutic information. Larger studies including other important markers are needed.

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