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Human Epidermal Growth Factor Receptor 2 and Estrogen Receptor Status in Respect to Tumor Characteristics in Non-Metastatic Breast Cancer

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Background: The expressions of estrogen receptor (ER) and cell surface receptor, Tyrosine Kinase Human Epidermal Growth Factor Receptor 2 (HER 2), have emerged as the most important molecular biomarkers determining the breast cancer prognosis. In this study, interactions between ER and HER2 were assessed to determine if they modulate tumor characteristics.

Materials and Methods: Tissue samples from 120 patients with early stage breast cancer receiving adjuvant chemotherapy were reviewed to evaluate ER and HER2 status quantified by immunohistochemistry and fluorescence in situ hybridization, and the correlation of ER and HER2 with patient characteristics and tumor pathology was studied.

Results: A total of 37(30.8%) and 80(66.6%) out of 120 samples were HER2 (3+ by immunohistochemistry or positive by fluorescent in situ hybridization) and ER positive (by immunohistochemistry), respectively. ER-negative tumors were significantly more likely to be HER-2 positive than were ER-positive tumors (21.25%; odds ratio, 0.270; 95% CI, 0.119 to 0.612; P=0.002). ER positivity was associated with <2 cm tumor size and higher histological grade (P=0.007 and 0.019, respectively). No significant correlation was seen between the co-expression of HER2 and ER and tumor characteristics.

Conclusion: HER2 positive tumors were less common compared to ER positive tumors in early stage breast cancer Iranian patients. Also, higher histological grade among ER negative tumors showed higher aggressiveness of the tumor. Future studies are needed to evaluate the effect of receptor status on prognosis.

Key words: Breast cancer, Tumor, Estrogen receptor, Human Epidermal Growth Factor Receptor 2 (HER2)

INTRODUCTION

Breast cancer is among the most common cancers affecting females worldwide (1-3). According to a report by the Iranian Ministry of Health and Medical Education in Iran, breast cancer ranks first among the malignancies affecting females (4). Some areas have a higher incidence of breast cancer such as East Africa (5) and the Middle East (including Iran) (6). In Iran, the incidence of breast malignancies is increasing. Patients are affected at a younger age and mostly detected at advanced stages (7, 8).

In breast cancer, determining the expression status of ER and cell surface receptor tyrosine kinas human epidermal growth factor receptor *(HER2/neu or c-erb-B2)* plays a critical role in choosing appropriate therapy (9). Estrogens potentially have mitogenic activity in normal

and cancerous breast tissues (10). Several studies have demonstrated this role in proliferation and progression of breast tumors by generating multiple growth-promoting signals (11-13). Evidence suggests that ER located on or near the cell membrane can activate HER2 (14).

Proliferation of breast tumoral cells and cell migration (15, 16) occur due to HER2 gene amplification and the relationships between HER2 and lymph node involvement, tumor size and grade have been documented (17). It seems that HER2 over-expression or amplification in tumor cells is associated with a poorer outcome (18).

The crosstalk between the ER and HER2 and the roll of HER2 in ER adjustment and balancing have been well known (19, 20). Some investigators suggest that HER2 activates multiple intracellular signaling pathways leading to ER regulation. In normal breast tissue, current activation causes estrogenic effect. In addition, ER actively contributes to this pathway by down-regulation (21) of HER2 expression and activation of intracellular pathways leading to increased HER2 activity. However, in breast cancer, when estrogen concentrations are low, activation of HER 2 may affect ER and increase tumor growth (22).

It was hypothesized that ER may act as a mediator in regulation of HER2 function. To the best of our knowledge, there are few studies regarding the relationship of ER with HER2 with respect to tumor characteristics in Iran. Thus, the results of this study can provide basic information on breast cancer in Iranian females, and may help predict patient prognosis.

MATERIALS AND METHODS

This survey was a retrospective single-institute study on 120 early stage breast cancer female patients referred to Iranmehr Hospital from August 1997 to January 2011. Written informed consent was obtained prior to patient enrollment in accordance with the guidelines of the medical ethics and scientific committees of Shahid Beheshti Medical University.

The study protocol was in compliance with the Declaration of Helsinki. Two anthracyclines-containing regimens were administered: CAF (n=28) vs. TAC (n=22) regimens. The administered doses were: 5 -fu 500 mg/m^2, Doxorubicin 50 mg/m^2, Cyclophosphamide 500 mg/m^2 in CAF (23) and Docetaxel 75mg/m^2, Doxorubicin 50 mg/m^2, Cyclophosphamide 500 mg/m^2 for TAC (24), which were repeated every 3 weeks.

Two-hundred files of breast cancer patients were reviewed and 120 cases were selected. The inclusion criterion was early stage breast cancer. The exclusion criterion was metastatic disease.

To determine the status of hormone receptors and HER2, immunohistochemical (IHC) methods alone (for ER and PR), or in combination with fluorescent in situ hybridization test (FISH) were used.

As recommended by the American Society of Clinical Oncology/College of American Pathologists (ASCO/CAP) (25) consensus panel and ESMO guidelines (26), first we assessed HER2 gene status by IHC. If IHC was 2+, the tumor block underwent confirmatory FISH test.

HER2 positivity was defined as samples with more than 10% of cells staining 3+ by IHC or 2+ by IHC along with FISH confirmation (a ratio of HER-2/neu gene/chromosome 17 ≥ 2.0). HER2 expression was determined by HerceptTest™ DAKO test. Breast cancer was classified according to the World Health Organization (WHO) classification of breast tumors.

In post-treatment follow-ups, patients underwent physical examination at least once every 4 months for the first 3 years, and every 6 months thereafter. Yearly mammograms, bone scans, and chest X rays were performed if necessary.

Statistical analysis

For testing the differences in categorical variables between the two groups, the chi-square test or Fisher's exact test was used. The difference in quantitative variables between the two groups was compared using the Student's *t*-test or non-parametric Mann-Whitney test. Estimated probabilities of HER2 positivity by significant factors were obtained from the models. Sensitivity and specificity of these models were derived, along with the receiver operating characteristic (ROC) curves, to assess how good the models were at predicting HER-2 positivity. All analyses were performed using SPSS version 21.

RESULTS

Tumor Pathology

One-hundred twenty patients were studied. Basic demographics of patients and pathological characteristics are shown in Table 1.

The mean age of menarche was 13.8 years. Malignancy was seen in the right breast in 47.6% of patients and the remaining had tumors in their left breast (no one had bilateral disease).

The median tumor size was 1 cm. Invasive ductal carcinoma was found to be the most frequent pathology. Modified radical mastectomy (MRM) and lumpectomy were performed for 88 and 32 patients, respectively. All patients received chemotherapy, and radiotherapy was performed in 56.7% of patients.

Association of HER2 positivity with other prognostic parameters

HER 2 over-expression was seen in 30.8 %(n=37) of the analyzed samples. All patients with over-expression of HER2 had invasive ductal carcinoma. The incidence of lymph node involvement was 51.1% among patients with known HER2 over-expression, vs. 43.2% in group without HER2 over-expression (P=0.237).

Association of ER expression with other prognostic parameters

A significant association was found between ER and tumor size (P=0.007). It means that large tumors were significantly more ER negative. Also, a significant correlation was seen between the histological grade and ER expression (P=0.019). However, given the ER status, no association was found between age, nuclear grade, lymph node involvement and menopausal status (Table 2).

Relationship between HER-2 Status and clinical and pathological variables

ER negative tumors were significantly more likely to be HER2 positive than were ER positive tumors (21.25%; odds ratio, 0.270; 95% CI, 0.119 to 0.612; *P* =0.002, Table 1). Thus, we selected the stepwise model including only the ER without the insignificant variables. The ROC curve from the reduced model is shown in Figure 1.

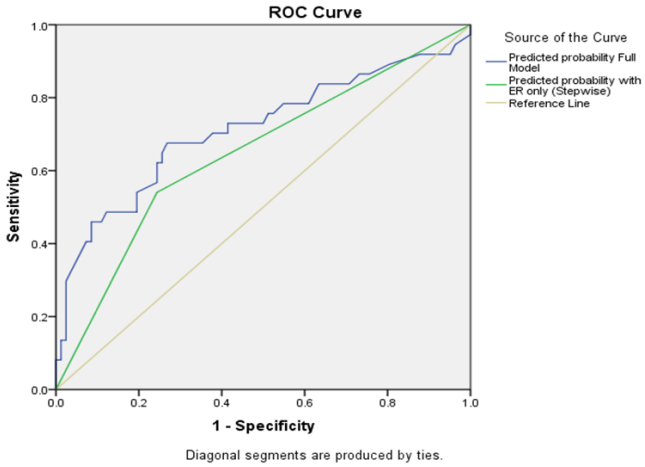

Figure 1. ROC curve from the reduced model.

Association of ER and HER2 with other prognostic parameters

The relation between pairs of assessed ER and HER2 showed no association between parameters' characteristics (Table 3).

Table 1. Clinicopathological characteristics of patients and association between HER2 and other parameters.

Age	All (n=120)	HER 2 Over expressed (n=37)	HER 2 non-Over expressed (n=83)	P value
Mean± SD	50.42±11.61	51.27±11.68	50.04±11.62	
Median	50.0	50.0	50.0	
IQR	44.0-58.7	46.50-59.0	42-58	
<40	24 (20.0%)	7 (18.9%)	17(20.4%)	0.981
40-49	33 (27.5%)	10 (27.0%)	23(27.71%)	
50-59	39 (32.5%)	13 (35.1%)	26(31.3%)	
>60	24 (20.0%)	7(18.9%)	17(20.4%)	
Histological grade				
I	29 (24.1%)	6(26.2%)	23(27.7%)	
II	42 (35%)	13(35.1%)	29(34.9%)	0.333
III	49 (40.8%)	18(48.6%)	31(37.3%)	
Nuclear grade				
0	7 (5.8%)	1 (2.7%)	6(7.2%)	
1	22 (18.3%)	3(8.2%)	19(22.8%)	0.137
2	63 (52.5%)	24(64.8%)	39(46.9%)	
3	28 (23.3%)	9(24.3%)	19(22.8%)	
Vascular invasion				
Absent	100(83.3%)	33 (89.2%)	67(80.7%)	0. 250
Present	20 (16.7%)	4 (10.8%)	16(19.3%)	
†Lymph node involvement				
None	66 (55.4%)	18 (48.6%)	48(57.8%)	
1 to 3	32(26.7%)	11 (29.7%)	21(25.3%)	0.237
4-9	17 (14.2%)	5 (13.5%)	12(14.4%)	
>9	4(3.3%)	3(8.1%)	1(1.2%)	
Unknown	1(0.8%)	0	1(1.2%)	
ER				
+	80 (66.7%)	17 (45.9%)	63(75.9%)	0.002*
-	40(33.3%)	20 (54.1%)	20(24.1%)	
‡Tumor size				
<2	27 (22.5%)	6 (16.2%)	21(25.3%)	0.486
2-5	83 (69.1%)	27 (73.0%)	56(67.4%)	
>5	10 (8.3%)	4 (10.8%)	6(7.2%)	
Pathology				
Invasive ductal carcinoma	111(92.5%)	37(86.04%)	74(89.1%)	0.144
Lobular carcinomas	6(5%)	0	6(7.2%)	
Others	3(2.5%)	0	3(3.6%)	
Menopausal status				
Yes	70(58.3%)	23(62.1%)	47(56.2%)	0.57
No	50(41.6%)	14(37.8%)	36(43.3%)	

Abbreviations: HER-2, human epidermal growth factor receptor 2; ER, estrogen receptor.

† No. of nodes involved: 0, node negative, 1 to 3:1 to 3 positive nodes, 4 to 9: 4 to 9 positive nodes; >9: >9 positive nodes.

‡ <=2cm: tumors less than 2 cm in size; 2-5cm: tumors between 2 and 4.99 cm in maximum diameter; >5 cm, tumors >5 cm in maximum diameter.

Table 2. Association of ER expression with other prognostic parameters.

Variables	ER positive (n=80)	ER negative (n=40)	P value
Age			
<40	12(15%)	12(30%)	
40-49	26(32.5%)	7(17.5%)	0.15
50-59	26(32.5%)	13(32.5%)	
>60	16(20%)	8(20%)	
Histological grade			
I	25(31.25%)	4(10%)	0.019*
II	28(35%)	14(35%)	
III	27(33.75%)	22(55%)	
Nuclear grade			
0	6(7.5%)	1(2.5%)	
1	16(20%)	6(15%)	0.449
2	42 (52.2%)	21(52.5%)	
3	16(20%)	12(30%)	
Unknown	0	0	
Vascular invasion			
Absent	65(81.25%)	35(87.5%)	0.386
Present	15(18.75%)	5(12.5%)	
†Lymph node involvement			
None	40(50%)	26(65%)	
1 to 3	22(27.5%)	10(25%)	0.27
4-9	13(16.3%)	4(10%)	
>9	4(6.25%)	0	
Unknown	0	0	
‡Tumor size			
<2	24(30%)	3(7.5%)	0.007*
2-5	52(65%)	31(77.5%)	
>5	4(5%)	6(15%)	
Pathology			
Invasive ductal carcinoma	74(92.5%)	37(92.5%)	0.885
Lobular carcinomas	4(5%)	2(5%)	
Others	2(2.5%)	1(2.5%)	
Menopausal status			
Yes	44(55%)	26(65%)	0.29
No	36(45%)	14(35%)	

Abbreviations: ER, estrogen receptor.

† No. of nodes involved: 0, node negative, 1 to 3: 1 to 3 positive nodes,4 to 9: 4 to 9 positive nodes; >9: >9 positive nodes .

‡ <=2cm: tumors less than 2 cm in size; 2-5cm: tumors between 2 and 4.99 cm in maximum diameter; >5 cm, tumors >5 cm in maximum diameter.

Table 3. Association of ER and HER2 with other prognostic parameters

	ER Positive (n=80)		P-value	ER negative (n=40)		P value
	HER2 non-over expressed(n=63)	HER2 over expressed(n=17)		HER2 non-over expressed(n=20)	HER2 over expressed(n=20)	
Age						
<40	8(12.6%)	4(23.5%)		9(45%)	3(15%)	0.95
40-49	21(33.3%)	5(29.4%)	0.288	2(10%)	5(25%)	
50-59	19(30.1%)	7(41.1%)		7(35%)	6(30%)	
>60	15(23.8%)	1(5.8%)		2(10%)	6(30%)	
Histological grade						
I	21(33.3%)	4(23.5%)	0.682	2(10%)	2(10%)	0.999
II	22(34.9%)	6(35.2%)		7(35%)	7(35%)	
III	20(31.7%)	7(41.1%)		11(55%)	11(55%)	
Nuclear grade						
0	5(7.9%)	1		1(5%)	0	
1	14(22.2%)	2	0.776	5(25%)	1(5%)	0.096
2	32(50.7%)	10(4.1%)		7(35%)	4(70%)	
3	12(19.04%)	4(1.6%)		7(35%)	5(25%)	
Vascular invasion						
Absent	50(79.3%)	15(88.2%)	0.406	17(85%)	18(90%)	0.633
Present	13(20.6%)	2(11.7%)		3(15%)	2(10%)	
†Lymph Node involvement						
None	33(52.3%)	7(41.1%)		15(75%)	11(55%)	
1 to 3	17(26.9%)	5(29.4%)	0.058	4(20%)	6(30%)	0.365
4-9	11(17.4%)	2(11.7%)		1(5%)	3(15%)	
>9	1(1.5%)	3(17.6%)		0	0	
Unknown	1(1.5%)	0		0	0	
‡Tumor size						
<2	19(30.1%)	5(29.4%)	0.349	2(10%)	1(5%)	0.597
2-5	42(66.1%)	10(58.8%)		14(70%)	17(85%)	
>5	2(3.1%)	2(11.7%)		4(20%)	2(10%)	

Abbreviations: HER-2, human epidermal growth factor receptor 2; ER, estrogen receptor.

† No. of nodes involved: 0, node negative, 1 to 3:1 to 3 positive nodes,4 to 9: 4 to 9 positive nodes; >9: >9 positive nodes,‡ <=2cm: tumors less than 2 cm in size; 2-5cm: tumors between 2 and 4.99 cm in maximum diameter; >5 cm, tumors >5 cm in maximum diameter.

DISCUSSION

Determination of factors, which may affect tumor characteristics and clinical behavior, can provide basic, important information on cancer. HER2 positive tumors were found to be less common (30.8%) compared to ER positive tumors (66.6%) and were inversely associated with ER positivity status (Table 1). Likewise, a significant association was found between ER and tumor size (Table 2). Also, a significant correlation was seen between ER negative tumors and high histological grade. In early stage breast cancer patients, data suggests that HER 2 status has a strong correlation with hormone receptors, especially ER.

In different studies, HER2 amplification was found in 20-30% of breast malignancies (27, 28); but in some

countries such as Lebanon a higher percentage was reported. HER2 overexpression in this study was in accordance with the data from Egypt and another study in Iran (29, 30).

We also confirmed that over-expression of HER2 was infrequent in invasive lobular cancers. However, our sample size was not large enough to exclude these cases from HER2 screening.

In several studies, nearly 50% of patients with HER2 amplification were also ER positive which is similar to the results of the present current study (31). Also, the data of our study were similar to those of other studies in that HER2 over expression in breast cancer was associated with ER-negative status (32,33). Amplification of HER2 oncogene is related to increased proliferation and cell migration (16,17).

Moreover, the expression ratio of HER2 and ER varies between different geographical regions. ER expression was seen in 66.6% of our patients, which was similar to a study by Bartlett et al, (20) and higher than the result of Moradi-Marjaneh et al (30). An insignificant correlation was found between younger age, larger size and higher nuclear and histological grade and ER negativity, which indicates worse prognosis. This result is similar to that of a report by Walker et al (34).

In relation to breast cancer biology, many parameters are known, but tumors expressing ER have a relatively favorable prognosis. Results of the current study showed that ER negative tumors had significantly higher histological grade than ER positive ones (Table 2); which may reflect higher aggressiveness of the tumor.

Some investigators have shown that only 10% of ER positive breast tumors at the time of diagnosis show HER2 over-expression but this rate was higher in our study (about 20); which may be due to the higher frequency of HER2 over expression (35).

It is assumed that the impact of HER2 on balancing ER, is applied via different, separate pathways such as RAS/MAPK or AKT/PI3Kinase (36, 37). ER may therefore modify the effect of HER2 expression on breast tumor pathology presumably via ER/HER2–mediated crosstalk. A number of potential pathways, which mediate this effect, are known and additional research may provide insight into the potential of this interaction to function as a therapeutic target. Considerations relative to ER and tumor differentiation provide a possible explanation for the dichotomy of response to adjuvant chemotherapy observed in pre- and postmenopausal women. We acknowledge the limitations of this study. First, this was strictly a single-institute investigation. Second, tumor grading, as well as tests for ER, PR and HER2, were performed by different laboratories without central supervision. Third, more than half the patients lacked information about tumor grading and vascular invasion, with the latter constituting the bulk of missing data.

This study was undertaken in early breast cancer patients and it would be useful to study this relationship more widely in other stages. Despite these shortcomings, our study is of value because 1) it highlights the importance of the ER and HER2 relationship and crosstalk between them; 2) it emphasizes the higher percentage of HER2 in our patients comparing to some countries as an important risk factor. Further research regarding the contribution of each of the tumor markers is underway with survival analyses adjusting for multiple risk factors.

Finally, the crosstalk between HER2 and ER status may help adopt multi-targeted strategies in the hope of improving patient outcome.

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