ORIGINAL ARTICLE

Survey of low ER-positive expression and its correlation with other clinical and pathological factors in breast cancer

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ABSTRACT

Introduction: Breast cancer is the most common cancer in women. Owning to the prominent role of biomarkers in molecular classification of breast cancer in recent years, evaluation of estrogen receptor (ER), progesterone receptor (PR), and Her2/neu seems to be required for prognosis and treatment of patients. **Material and Methods:** One-hundred twenty two patients with primary breast carcinoma were selected and immunohistochemistry staining for ER, PR, and Her2/neu were performed on representative paraffin blocks. ER level can be semi-quantified by immunohistochemistry using the H-score. The score, given as the sum of the percent of tumor cells staining multiplied by the intensity level, ranges from 0 to 300 as low, intermediate, and high grades. The statistical association of ER expression with the level of PR and Her2/neu, tumor size, necrosis, microscopic grade, vascular invasion, and lymph node involvement were analyzed using SPSS16 software. **Results:** Results showed that among 122 studied patients, 44.3% were in the low ER-positive group where most of these cases (22.1%) were Her2/neu negative. Although there was a reciprocal interplay between the expression of ER and Her2/neu, increased expression of ER had a direct relation with PR level. However, there was no statistical relation between ER level with age, tumor size, necrosis, microscopic grade, vascular invasion, and lymph node involvement. **Discussion:** The study clearly indicated that low ER group encompasses the high frequency of breast cancer patients. Furthermore, the most cases of low ER patients were in Her2/neu negative group.

KEY WORDS: Breast cancer, estrogen receptor, Her2, immunohistochemistry, progesterone receptor

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INTRODUCTION

Breast cancer is the most common malignancy in women with incidence of one million cases in a year.[1] Breast cancer is the second most common cause of cancer death among 20--59 years old women after lung cancer.[1,2] The prevalence of breast cancer in developed countries (except Japan) is more than developing countries that is presumably related to the lifeway and dietary regimens of women.[3] Despite the increasing incidence of breast cancer, the mortality of patients is decreased due to early diagnosis and well-documented therapeutic procedures.[1]

One of the most important classifications of breast cancer is based on the expression of estrogen receptor (ER), progesterone receptor (PR), and Her2/neu receptor. Immunohistochemically, 50--85% of breast cancers are positive for ER that is more common in menopause patients.[4,5] Given that estrogen is able to induce the expression of progesterone, it seems that there is an indispensable concordance between the expression of ER and PR markers. In breast cancers, high frequency of tumors are positive for both ER and PR and just about 10% of tumors with PR positive is ER negative.[6] Positivity for hormone receptors (ER and PR) in breast cancer is associated with diminished relapse and increased sensitivity to hormone therapy,

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so that about 80% of positive for ER/PR (ER or PR) and less than 10% of negative for ER/PR respond to treatments. Undoubtedly, the expression of ER is a crucial biomarker in breast cancer because of susceptibility index to endocrine therapies. ER-positive tumors are the main target of endocrine therapy.[7] There is accumulating evidence that the expression of PR is considerably attributed to ER expression. Tumors with overexpression of PR and negative for ER are rare and encompass less than 1% of breast cancer.[8] Overexpression of PR is assessed by nuclear staining using relevant antibodies through immunohistochemistry (IHC).

Epidermal growth factor receptor (Her2/neu) is an oncogene that encoded a transmembrane glycoprotein with tyrosine kinase activity belongs to the family of epidermal growth factor receptors.[9] Her2/neu overexpression is found in about all cases of high grade ductal carcinoma *in situ* (DCIS), 20-30% of cases of invasive ductal carcinoma, and smaller percentage of invasive lobular carcinoma.[5] In some cases of breast cancer, the expression of ER, PR (hormone receptors), and Her2/neu is negative that is known as triple negative breast cancer.[4] Expression of Her2/neu (C-erb-B2) is considered as a prognostic indicator of breast cancer.[10-12] It has been suggested that overexpression of Her2/neu is resulted in overall poor survival. Overexpression and amplification of Her2/neu was analyzed by IHC, fluorescent *in situ* hybridization (FISH), and chromogenic *in situ* hybridization (CISH) methods.[13] However, the first approach is IHC that its results were reported as 0, +1, +2, and +3. The scores of 0 and +3 are related to 0 and 100% of Her2/neu expression, respectively. But scores of +1 and +2 should be checked by FISH to make final decision on the expression of Her2/neu. Frozen tissues fixed in formalin and cytological smears fixed in alcohol were used to evaluate Her2/neu expression.[14]

To sum up, the aim of the present study was to determine the status of low ER-positive breast cancers and its association with a number of clinical and pathological features. Results showed that the expression of ER in low ER-positive breast cancers were in association with the expression of PR and HER2/neu.

MATERIALS AND METHODS

Materials

Monoclonal antibodies of ER, PR, and Her2/neu, Biotin Blocking System, Liquid DAB + Substrate Chromogenic System, EnVision + Dual Link System, Target Retrieval Solution were provided from DAKO (Denmark). Hematoxylin was purchased from Panreac (Spain). Hydrogen peroxide, methyl alcohol, Entelan glue, and ethyl alcohol 99.6% were supplied from Merck (Germany). Pepsin was provided from Sigma (Germany). Xylene and ethyl alcohol (96--70%) were purchased from shimi-nab (Iran).

Patient samples

In this retrospective study, achieved paraffin-embedded tissues were retrieved from patients diagnosed as breast cancer from 2012 to 2016. Of these, 122 cases of breast carcinoma were identified. The slides prepared from paraffin-embedded specimens were stained with the conventional hematoxylin and eosin method. All specimens including needle biopsies and mastectomies were reviewed and original diagnoses were confirmed by pathologist and pathology resident.

Hematoxylin and eosin (H and E) staining

Paraffin-embedded tissues were provided as 4 μm sections and stained with hematoxylin and eosin (H and E) method. In this method, glass slides containing tissue sections incubated at 70°C for 2 h. Then, slides rinsed into several jars filled with xylene, graded series of ethanol solutions, hematoxylin, lithium carbonate, and eosin. Stained sections were independently evaluated by two pathologist and pathology resident.

Immunohistochemistry

Immunohistological staining was performed on formalin-fixed paraffin-embedded tissue sections using antibodies against ER, PR, and Her2/neu. For this aim, 4 μm tissue sections were deparaffinized at 37°C for 2 h and xylene for 24 h. Then, slides rehydrated in a graded series of ethanol solutions and PBS (phosphate buffered saline) for about 12 min. To retrieve antigens, slides immersed in the jar containing Tris buffer (pH = 9) and heated in water bath at 95°C for 20 min followed by washing in PBS solution. To quench the intracellular activity of peroxidases, slides were immersed in a solution of 3% hydrogen peroxide in methanol for 10 min, washed with PBS, and placed in jars containing avidine solution for 5 min. Then, biotin was added to increase the specificity of staining. After washing with PBS, slides were incubated by primary and secondary antibodies for 45°C and 30°C, respectively, in a humid and dark place at room temperature. The slides were washed in PBS and stained with substrate–chromogen solution known as 3,3'-diaminobenzidinetetrahydrochloride (DAB) for 5 min. The counterstaining was performed with hematoxylin for 30 s and washed in water. The stained slides immersed in graded series of ethanol and then, xylene to transparence, and dehydration of tissues. Then, slides mounted to study under microscope. Negative controls were exposed to antibody diluent replacing primary antibody. The intensity of immunohistochemical staining was evaluated and degrees of differentiation (well, moderate, and poor) were assigned to respectively, as grades of 1 to 3 based on H-scoring level. H-scoring method was expressed as two parameters including the total number of stained tumor cells and the intensity of staining as follow:

$$\text{H - score} = \%\ \text{stained tumor cells} \times \text{intensity of staining}$$

For assessment of H-score, 10 randomly fields under microscope was chosen to determine the scores of 0, 1, 2, and 3 corresponding to the absence, weak, moderate, and strong nuclear staining intensity, respectively. In this line, H-score 300 was considered as high expression (100% stained tumor cells ×3), while H-score between 0 and 100 is related to low ER positivity.[13] Besides, PR and HER2/neu receptors were evaluated by IHC. Classification

of PR was performed based on positive (more than 1% nuclear staining for PR) and negative (less than 1% staining).

Statistical analyses

Data were analyzed using *t*-test and Chi-square in SPSS (V.16).

RESULTS

Women patients included 121 cases with invasive ductal carcinoma and one case with invasive lobular carcinoma. Age range of patients was 26--78 years with a median age of 49.5 years. The median tumor size was 3.5 cm (range 0.6--9 cm). Among 122 breast cancer patients, 44.3% (54 cases), 28.7% (35 cases), and 27% (33 cases) were stained negative, low, and intermediate for ER expression, respectively [Figure 1 and Table 1]. There is no high expression of ER and most cases were negative for ER receptor. No meaningful association was shown between the type of ER group and the age of patients (P value = 0.08). In both age groups (<50 and ≥50), most people were in the group of low ER (44.3%). Table 2 was summarized the overall characteristics of studied patients.

Most of the patients (both in low ER positive and also in two other groups) reveal "infiltrating ductal carcinoma" subtype. Proliferative activity (i.e., Ki67 immunostaining) is not performed. In addition, there was no significant relation between ER group and microscopic grade of breast cancer (P value = 0.136) based on Fisher statistical test.

Table 1: The frequency of patients based on ER expression using H-score method

Estrogen Receptor (H-SCORE)	*Number (%)*
Low	54 (44.3%)
Intermediate	33 (27%)
Negative	35 (28.7%)

It is evident that 77.3% of ER group showed positivity for vascular invasion in which low ER group had the highest positivity for vascular invasion. In addition, no significant meaningful relation was observed between vascular invasion and ER expression based on χ^2 statistical method (P value = 0.930).

The appearance of ER groups in breast cancer specimens was also evaluated based on necrosis. Results showed that nearly 80.8% of specimens were negative for necrosis. According to statistical analysis, there was no meaningful association between ER group and necrosis (P value = 0.565).

The incidences of low ER-positive group in breast cancer patients based on involvement of lymph node showed that the percentage of negative and positive lymph nodes were about 18.3% and 28.8%, respectively. Besides, there was no significant relation between ER groups and lymph node involvement based on χ^2 statistical test (P value = 0.666). It should be mentioned that lymph node involvement was not determined in 18 cases, so they did not consider in statistical analysis.

Given that 1 case of lobular carcinoma was found among patients, the magnification of ER group was surveyed based on the type of lobular and ductal breast carcinoma [Table 2]. The exact fisher test showed no significant relation between ER group and the type of tumor (P value = 1).

Her2 receptor is an important marker in classification of breast cancer. The difference between Her2/neu

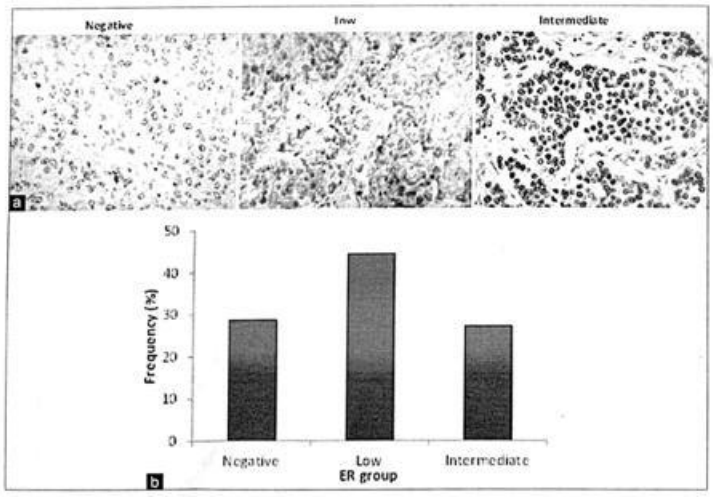

Figure 1: Staining of PR receptor in breast cancer tumors. Immnunohistochemical staining of breast cancer tissues against PR (a). Frequency of PR classification based on H-score method (b)

Table 2: The appearance incidence of ER groups based on the several varieties of breast cancer

	ER Group			Total
	Negative	Low ER	Intermediate	
Microscopic grade				
Not identified	0	0.8%	2.5%	3.3%
Grade 1	7.4%	11.5%	9%	27.9%
Grade 2	17.2%	31.1%	13.1%	61.5%
Grade 3	4.1%	0.8%	2.5%	7.4%
Age				
<50	14.8%	27.9%	13.9%	56.6%
≥50	13.9%	16.4%	13.1%	43.4%
Vascular invasion				
Negative	5.9%	10.9%	5.9%	22.7%
Positive	22.7%	34.5%	20.2%	77.3%
Necrosis				
Negative	22.5%	35.8%	22.5%	80.8%
Positive	6.7%	9.2%	3.3%	19.2%
Lymph node involvement				
Negative	12.5%	18.3%	11.5%	42.3%
Positive	17.3%	28.8%	11.5%	57.7%
Diagnosis				
Ductal	28.7%	43.4%	27%	99.2%
Lobular	0	0.8%	0	0.8%
Tumor size (mm)				
<20	9.1%	10.9%	5.5%	25.5%
20-50	13.6%	24.5%	18.2%	56.4%
>50	5.5%	9.1%	3.6%	18.2%
Her2/neu				
Negative (0-1)	17.2%	22.1%	13.1%	52.5%
Weakly positive (2)	5.7%	19.7%	12.3%	37.7%
Strongly positive (3)	5.7%	2.5%	1.6%	9.8%
PR				
Negative	19.7%	6.6%	9%	35.2%
Positive	9%	37.7%	18%	64.8%

positivity and ER positivity is statistically meaningful (P value = 0.044). Among 122 patients, 52.2% of cases were negative (grade of 0--1) which 22.1% of them were in low ER group. In the group of weakly positive HER2/neu immunohistochemistry staining (grade 2) (37.7%), the most cases (19.7%) were low ER group. In Her2/neu with strong positive staining (grade3) (9.8%), the majority were negative for ER (5.7%), indicating there was a reciprocal relationship between the appearance of Her2/neu and ER. Increasing appearance of Her2/neu was associated with decreasing occurrence of ER (enhancement of negative ER).

Another clinical prognostic marker for breast cancer is PR receptor. The frequencies of positive and negative PR groups were 64.8% and 35.25%, respectively. According to χ^2 test, there was a significant relation between the appearance of ER and PR receptors (P value <0.001). Among 122 patients, the bulk of population was PR positive (64.8%) in whom the majority was in low ER group (37.7%). Results showed a direct interplay between the magnification of ER and PR receptors with meaningful statistical relationship.

The appearance of ER was also studied based on the size of tumor. There was no significant difference between ER appearance and the size of tumors (P value = 0.9) based on Kruskal--Wallis test. The range of tumor size was 0.6--9 cm with average size of 3.5 cm, the majority of patients (56.4%) were the range of 2--5 cm tumor size, the second group was the range of <2 cm (25.5%), and tumors with the size of >5 cm were the least group (18.2%). In low ER group, the most of tumors were in the range of 2--5 cm.

DISCUSSION

The purpose of this study was to investigate the prevalence of low ER positive and its association with certain clinical and pathological properties in breast cancer. Breast cancer is a heterogeneous disease clinically and biologically. In this line, several clinical and tissue-specific factors are involved in determining prognosis and even therapy including age, lymph node involvement, tumor size, the type of tumor, tumor grade, apoptosis, differentiation degree, and vascular invasion.[4] For instance, the prognosis of breast cancer is highly variable and is influenced by key factors such as age, tumor size, tumor degree, vascular invasion, lymph node involvement and above all, molecular markers. Molecular markers were extensively used to recognize the type of tumor and anticipate the response to therapy. Therapeutic strategies include endocrine remedies for ER-positive tumors, Herceptin for Her2/neu positive tumors and chemotherapy for triple negative breast tumors that does not have any specific targets. Among molecular markers, ER, PR, and Her2 have the most importance because of their role in prognosis and treatment of patients.

As shown in results, 71.3% were positive ER and 28.7% were negative for ER. Among ER-positive group, 44.3% and 27% of population had low and intermediate grades, respectively, according to H-score method. These results are in concordance with the study performed with NikaC, Gloyeske, and David j. Dabbs in which about 70--75% of patients was positive for ER. This study is the based on H-score method and the results is similar to our study.[15] Another study was performed with Shahin Sayed and Zahir Moloo, of 304 patients in this study, 72.4% were ER positive.[3] The data of this study was line with our results. Among PR-positive patients, the majority was also positive for ER, whereas PR-negative population has the negative for ER, indicating the direct relationship between two markers. Gloyeske *et al.* showed there is a meaningful relation between ER and PR,[15] while in the research by Yu *et al.* there was no meaningful association.[16] In another study with 72.4% ER-positive patients, 64.8% of patients were also positive for PR that had a similar association with our study.[3] The results showed that there was a meaningful association between ER and the other important biomarkers. In fact, overexpression of Her2/neu (Her2/neu+) was associated with high frequency of ER-negative population and there was a reciprocal relation between the expression of ER and Her2/neu. Although the majority was Her2/neu positive in other studies, there was no meaningful association between Her2/neu and ER markers.[3,16]

Although 77.3% of population was positive for vascular invasion, the association between ER and vascular invasion was not shown in the present study. The positivity of vascular invasion was shown to be prevalent in low ER group respective to the group with intermediate expression of ER. Furthermore, the higher frequency of vascular invasion in negative ER than intermediate ER indicates that the expression of ER has not a meaningful association with vascular invasion. In this line, we proposed that since the used procedure to evaluate vascular invasion based on H and E staining may lead to the poor interpretation, other methods such as D2-40 immunostaining is required to accurate interpret the invasion to vascular tissue. Our results also showed no meaningful association between the expression of ER with age, tumor size, and involvement of lymph node that confirm the results of other studies in these fields.[3,16]

CONCLUSION

Our study reveals that of the 121 patients, the low ER group has constituted the prevalence rate (44.3%) with high frequency of Her2/neu negative cases (22.1%). In addition, ER expression had a direct relation with PR, reciprocal association with Her2/neu, and no association with other clinical features of tumor. This investigation can provide a valuable implication to identify the most important prognostic biomarkers in breast cancer and their application to manage the patients.

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Conflicts of interest

There are no conflicts of interest.

REFERENCES

1. Guinee V. Epidemiology of breast cancer. Breast 1998;1:339-51.
2. Jemal A, Siegel R, Ward E, Hao Y, Xu J, Murray T, *et al*. Cancer statistics, 2008. CA Cancer J Clin 2008;58:71-96.
3. Sayed S, Moloo Z, Wasike R, Bird P, Oigara R, Govender D, *et al*. Is breast cancer from Sub Saharan Africa truly receptor poor? Prevalence of ER/PR/HER2 in breast cancer from Kenya. Breast 2014;23:591-6.
4. Robbins S, Kumar V, Abbas A. Cotran Pathologic Basis of Disease. Philadelphia: Saunders; 2010.
5. Rosai J. Rosai and Ackerman's Surgical Pathology. China: Elsevier Health Sciences; 2011.
6. Dabbs DJ. Diagnostic Immunohistochemistry. United States of America, Philadelphia: Elsevier Health Sciences; 2013.
7. Bauer KR, Brown M, Cress RD, Parise CA, Caggiano V. Descriptive analysis of estrogen receptor (ER)-negative, progesterone receptor (PR)-negative, and HER2-negative invasive breast cancer, the so-called triple-negative phenotype. Cancer 2007;109:1721-8.
8. Viale G, Regan MM, Maiorano E, Mastropasqua MG, Dell'Orto P, Rasmussen BB, *et al*. Prognostic and predictive value of centrally reviewed expression of estrogen and progesterone receptors in a randomized trial comparing letrozole and tamoxifen adjuvant therapy for postmenopausal early breast cancer: BIG 1-98. J Clin Oncol 2007;25:3846-52.
9. Bazley L, Gullick WJ. The epidermal growth factor receptor family. Endocr Relat Cancer 2005;12(Suppl 1):S17-27.
10. Pegram MD, Konecny G, Slamon DJ. The molecular and cellular biology of HER2/neu gene amplification/overexpression and the clinical development of herceptin (trastuzumab) therapy for breast cancer. Advances in Breast Cancer Management. New York: Springer; 2000. p. 57-75.
11. Mass RD, Press MF, Anderson S, Cobleigh MA, Vogel CL, Dybdal N, *et al*. Evaluation of clinical outcomes according to HER2 detection by fluorescence *in situ* hybridization in women with metastatic breast cancer treated with trastuzumab. Clin Breast Cancer 2005;6:240-6.
12. Slamon DJ, Clark GM, Wong SG, Levin WJ, Ullrich A, McGuire WL. Human breast cancer: Correlation of relapse and survival with amplification of the HER-2/neu oncogene. Science 1987;235:177-82.
13. Wolff AC, Hammond MEH, Schwartz JN, Hagerty KL, Allred DC, Cote RJ, *et al*. American society of clinical oncology/college of American pathologists guideline recommendations for human epidermal growth factor receptor 2 testing in breast cancer. J Clin Oncol 2006;25:118-45.
14. Weigel MT, Dowsett M. Current and emerging biomarkers in breast cancer: Prognosis and prediction. Endocr Relat Cancer 2010;17:R245-62.
15. Gloyeske NC, Dabbs DJ, Bhargava R. Low ER+ breast cancer: Is this a distinct group? Am J Clin Pathol 2014;141:697-701.
16. Yu L, Ke W, Wang Y, Ding W, Wang B, Huang S, *et al*. Predictive and prognostic value of ER-α36 expression in breast cancer patients treated with chemotherapy. Steroids 2014;84:11-6.