Relation of Estrogen Receptor Expression to Recurrence and Survival in Early and Locally Advanced Breast Cancer

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Abstract

Background: Breast cancer is the most diagnosed cancer among women worldwide, accounting for almost 1 in 4 cancer cases. It is the second most frequent and the leading cause of death from cancer in women. In breast cancer, assessment of hormonal receptor status is fundamental having both prognostic and predictive role. The studies that focused on the impact of estrogen receptor (ER) expression showed controversial results. So in this study our aim was to determine the effect of ER expression on disease recurrence and survival in non-metastatic breast cancer patients.

Patients and methods : This is a retrospective study including 110 non metastatic breast cancer patients with the exclusion of human epidermal growth factor receptor 2 (Her2) positive cases starting from January 2006 to December 2010. Patients were divided into three groups hormonal negative (0%), low/ intermediate (1-50%) and strong (51-100%) ER expression according to the quantitative ER measurement.

Results: There was a positive correlation between the level of ER expression and overall survival (OS) which was not true for disease free survival (DFS).

Conclusion: The relation between the level of ER expression and survival was significant while there was no significant relation to disease recurrence.

Key words: Estrogen receptor, breast cancer, survival, recurrence.

Introduction:

Breast cancer is the most commonly diagnosed cancer among women worldwide and the leading cause of cancer death in women. The stage at which women are diagnosed with breast cancer now has been shifted due to increased public awareness and screening programs. About 75% of newly diagnosed cases are classified as early breast cancer [1]. The estrogen receptor (ER) was first identified in the late 1960s, it is a protein molecule located in the nuclei of hormone target cells. The ER receptor binds to estrogen and structurally similar ligands [2].

The discovery of ER receptor was a major breakthrough in the management of breast cancer partly as a prognostic factor and also predictor of response to endocrine therapy, the first reports about its role emerged in the 1970s [3].

It's been desired to make endocrine therapy feasible for all hormonal positive patients but there's al-ways been a concern about the benefit gained by patients which would not be equal and there isn't a consistent cut-off level for high and low levels of expression below which there would be little benefit from endocrine therapy [4].

In clinical practice immunohistochemistry (IHC) is considered the gold standard in determining the level of ER expression as a product of the percentage of epithelial cells stained and the intensity of staining (4).

Historically the cut-off level for ER positivity was 10% or more nuclear staining of epithelial compo-nent of the tumor however tumors with even 1-10% staining which is considered weak gained clinical benefit from hormonal therapy [5].

Theoretically it's predicted that the stronger the expression of ER and /or progesterone receptor (PR) the better the response to endocrine therapy. It was proposed by St. Gallen Expert Consensus Confer-ence on Primary Therapy of Early Breast Cancer in 2017, that hormonal positive early breast cancer tumors are of low risk but there wasn't any proposed cut-off to determine high and low levels of expression [6].

The presence of hormonal receptors (HRs) on invasive breast cancer cells is both prognostic and predictive, although it seems to be more predictive. The presence of these receptors confers a reduced risk of

recurrence and death within 5 years of diagnosis. The presence of HRs is also predictive of response to adjuvant endocrine therapy. Further prediction of response to endocrine therapy is established by subdividing HR status into ER positive and PR positive subgroups [7].

During the year 2010 the American Society of Clinical Oncology and the College of American Pathologists announced guidelines stating that ≥1% of tumor nuclei with positive stain for ER should be the cutoff point for ER positivity. The panel drew attention towards the lack of studies assessing the quantitative measurement of ER stain to make correlation of such measurements with prognosis and outcome and predict response to hormonal therapy [8].

An interesting study reported by Cuzick and colleagues in 2011 done on postmenopausal women with ER-positive breast cancer concluded that the quantitative ER (H)-score alone or in combination with the other three markers; PR, human epidermal growth factor receptor 2 (Her2) and Ki-67 was associated with risk of distant recurrence [9].

Aim of the study:

Identification of the distribution of ER expression among early and locally advanced invasive ductal carcinoma (IDC) breast cancer patients, determining the correlation between clinicpopathologic characteristics and the level of ER expression, and the impact of the level of ER expression on disease free survival (DFS) and overall survival (OS).

Patients and Methods:

This study is a retrospective study aiming to assess the relation between the level ER expression and breast cancer recurrence and mortality. Approval was obtained from the research ethics committee of South Egypt Cancer Institute (SECI), Assiut University with Institutional Research Board (IRB) number: 48.

The records of all female patients with non metastatic breast cancer presented at med-ical oncology department SECI from January 2006 to December 2010 were included in the study after application of the eligibility criteria of the proposed protocol.

Inclusion criteria

All patients who were ≥18 years of age, non metastatic histologically confirmed (IDC), no specific type (NST) were included in the study.

Exclusion criteria

Patients diagnosed as breast cancer of any pathological type other than IDC, NST, metastatic breast cancer at diagnosis, Her2 status positive or unknown, patients whose paraffin blocks were not available and patients with history of other malignancy were excluded from the study.

Data Collection

Medical records of the patients were extracted from the archive of Medical Oncology Department, Radiotherapy Department, Pathology Department at SECI and Clinical Oncology Department, Assiut University.

Collected data included clinicopatholoical characteristics and treatment plans, including surgery, chemotherapy, radiotherapy, and endocrine therapy. Available information regarding ER and PR (percent of tumor cells staining positive for each marker by (IHC).

Pathology methodology

An experienced pathologist re-reviewed the Hematoxylin and eosin (Hx&E) stained slides to confirm the diagnosis IDC, NST. Tissue IHC was used to determine ER expression. For cases whose percent of ER positive tumor cells were not recorded, the immunostained slide for ER was extracted from the archive of Pathology Department and re-examined by the pathologist. When the immunostained slide was not available, its Paraffin block was extracted and 4 um section thickness was cut on a positively charged slide and stained for ER using the standard methods of DAKO laboratories and assessed for the percent of nuclear staining of tumor cells. (Figure1).

The score used for the detection of ER expression was transformed to a percentage so patients were categorized into three groups; negative ER expression if 0% staining, low/intermediate ER expression from 1% to 50% staining and strong ER expression when equal or above 51% staining [10].

Follow up

After termination of treatment, patients were followed up every three months for the first two years and every six months for 5 years and yearly thereafter by physical examinations and laboratory studies and mammography which was done annually. Local recurrence was proved by biopsy. Radiological studies including computerized tomography or bone scans were done if clinical symptoms and signs indicated visceral or bone metastasis.

Statistical analysis

Data were verified, coded by the researcher and analyzed using IBM-SPSS 21. Descriptive statistics: Means, standard deviations, medians, ranges and percentages were calculated. Test of significances: Chi square test was used to compare the difference in distribution of frequencies among different groups. For continuous variables; independent t-test analysis was carried out to compare the means. Kaplan–Meier curve was used to estimate the median survival time. The Log-rank test was used to com-pare survival curves between the categories of the explanatory variables. A p-value equals or less than 0.05 was considered significant [11].

Results:

One hundred and ten female patients were eligible for enrollment in the study with a median age of 50 years ranging from 27 to 82 years. Sixty three (57.3%) patients were postmenopausal and 42 (38.2%) patients were classified as T2. Twenty two (20%) patients had

N2 nodal stage, (Table 1). Patients were categorized into three groups according to the level of (ER) expression (Fig.1). First group with negative ER expression included 19 patients, 53 in the second group having ER expression from 1% to 50%, the third group 38 patients with ER expression from 51% to 100%. (Table1).

Treatment plans:

Treatment plans were approved by weekly institutional tumor board.

One hundred patients underwent surgery either modified radical mastectomy (MRM) in 75 patients (68.2%) or breast conservative surgery (BCS) in 35(31.8%) patients which included lumpectomy, wide local excision, quadrantectomy, or segmental resection. Re-excision was performed if margins were positive. Levels I and II axillary lymph nodes were dissected and level III was dissected in case of suspected nodal involvement of level II.

Systemic chemotherapy was administered as indicated which consisted of at least four cycles of anthracycline and /or Taxane based chemotherapy.

Twenty five patients (22.7%) received neoadjuvant chemotherapy while seventy patients 70 (64%) patients received adjuvant chemotherapy.

Ninety five patients (86.4%) with hormonal positive disease received hormonal therapy, 45 (40.9%) premenopausal patients received tamoxifen and 50 (45.5%) postmenopausal patients received aromatase inhibitors, only15 (13.6%) patients had both negative ER and PR.

Adjuvant radiotherapy was offered to 55 (50%) patients including all patients who underwent BCS and those who underwent MRM with tumors ≥4 cm or pathologically involved axillary lymph nodes. Postoperative radiotherapy was delivered to the breast and/or chest wall using tangential fields and matched with the direct supraclavicular field when indicated. Patients were scheduled on conventional fractionation at a dose of 50 Gy/25 fractions (2.0 Gy/fraction). A boost dose of 14 Gy in 7 fractions to the tumor site using 12 Mev electrons prescribed at the 90% isodose line was given to all patients.

Treatment outcome

The median OS of the whole study group was 61 months and the median DFS was 37 months. Patients in the first group with negative hormonal receptors had a median OS of 47 months, patients of the second group with low/intermediate ER expression (1-50%) had a median OS of 58 months and the third group with strong ER expression (51-100%) had 73 months median OS (Figure 3).

A total of 46 patients experienced disease recurrence, the first group of patients with negative ER expression 11 out of 19 patients experienced recurrence with a median DFS of 41 months, in the second and third groups 22 out of 53 patients and 13 out of 38 patients experienced disease recurrence and their median DFS was 32 and 44 months respectively (Figure 4).

There was a direct relation between the level of ER expression and OS with p value = 0.014, however was no correlation between the level of ER expression and disease recurrence with p value = 0.540. There was a significant relation between the level of ER expression and T & N staging with p value = 0.025 and 0.048 respectively. This was also evident in PR expression with p value <0.001 (Table 2).

Table 1: Descriptive Statistics of the study group

Variable	Category	
Number		110
Age in years	Mean ± SD	50.18±11.2
	Median (range)	50 (27.82)
Menopausal Status	- Pre-	47 (42.7%)
	- Post	63 (57.3%)
T-staging	- Tx	23 (20.9%)
	- T0	1 (0.9%)
	- T1	16 (14.5%)
	- T2	42 (38.2%)
	- T3	17 (15.5%)
	- T4	11 (10.0%)
N-staging	- Nx	20 (18.1%)
	- N0	28 (25.5%)
	- N1	34 (30.9%)
	- N2	22 (20.0%)
	- N3	6 (5.5%)
Level of ER expression	- 0%	19 (17.3%)
	- 1-50%	53 (48.2%)
	- 51-100%	38 (34.5%)
PR expression	- Negative	30 (27.3%)
	- Positive	80 (72.7%)
Grade	- G1	2 (1.8 %)
	- G2	96 (87.3%)
	- G3	12 (10.9%)
Lymphovascular invasion	- Absent	90 (81.8%)
	- Present	20 (18.2%)
Surgery	- BCS	35(31.8%)
	- MRM	75(68.2%)
Chemotherapy	- Neoadjuvant	25 (22..7%)
	- Adjuvant	70 (64.0%)
Hormonal therapy	- Adjuvant	95 (86.4%)
Radiotherapy	- Adjuvant	55 (50.0%)

N; Number, **SD;** Standard deviation, **T staging;** Tumor staging, **N staging;** Nodal staging, **ER;** Estrogen receptor, **P**R; Progesterone receptor, **BCS;** Breast conservative surgery, **MRM;** Modified radical mastectomy.

Table 2: Clinical Characteristics of the Study group in relation to ER Expression

Parameter	All (n=110)	ER expression (0%) Negative (n = 19)	(1-50%) Low/intermediate (n = 53)	(51-100%) Strong (n = 38)	P-value
Age/years					= 0.950*
Mean ± SD	50.18 ± 11.2	49.58 ± 9.4	50.11 ± 11.7	50.58 ± 11.5	
Median (Range)	50 (27 - 82)	49 (33 - 66)	50 (27 - 82)	50 (30 - 75)	
P-value**		1 vs 2 = 0.860	2 vs 3 = 0.846	1 vs 3 = 0.753	
Menopausal Status					= 0.359***
• Pre-	47 (42.7%)	8 (42.1%)	24 (45.3%)	15 (39.5%)	
• Post-	63 (57.3%)	11 (57.9%)	29 (54.7%)	23 (60.5%)	
Grade					= 0.194****
• I	2 (1.8%)	0 (0%)	1 (1.9%)	1 (2.6%)	
• II	94 (85.5%)	14 (73.7%)	45 (84.9%)	35 (92.1%)	
• III	14 (12.7%)	5 (26.3%)	7 (13.2%)	2 (5.3%)	
T-Stage					**= 0.025****
• T0	1 (0.9%)	0 (0%)	0 (0%)	1 (0%)	
• T1	16 (14.5%)	2 (10.5%)	2 (3.8%)	12 (31.6%)	
• T2	42 (38.2%)	10 (52.6%)	24 (45.3%)	8 (21.1%)	
• T3	17 (15.5%)	2 (10.5%)	9 (17%)	6 (15.8%)	
• T4	11 (10%)	2 (10.5%)	5 (9.4%)	4 (10.5%)	
• TX	23 (20.9%)	3 (15.8%)	13 (24.5%)	7 (18.4%)	
N-Stage					**= 0.048****
• NX	20 (18.2%)	0 (0%)	11 (20.8%)	9 (23.7%)	
• N0	28 (25.5%)	7 (36.8%)	13 (20.8%)	8 (21.1%)	
• N1	34 (30.9%)	5 (26.3%)	19 (24.5%)	10 (26.3%)	
• N2	22 (20%)	4 (21.1%)	8 (15.1%)	10 (26.3%)	
• N3	6 (5.5%)	3 (15.8%)	2 (3.8%)	1 (2.6%)	
PR Status					**< 0.001*****
• Negative	44 (40%)	17 (89.5%)	27 (50.9%)	0 (0%)	
• Positive	66 (60%)	2 (10.5%)	26 (49.1%)	38 (100%)	
Recurrence					= 0.540***
• No	64 (58.2%)	8 (42.1%)	31 (58.5%)	25 (65.8%)	
• Yes	46 (41.8%)	11 (57.9%)	22 (41.5%)	13 (34.2%)	
Death					**= 0.014*****
• No	52 (47.3%)	2 (10.5%)	27 (50.9%)	23 (60.5%)	
• Yes	58 (52.7%)	17 (89.5%)	26 (49.1%)	15 (39.5%)	

*ANOVA test was used to compare the mean difference between groups.
**Post-hoc test with Bonferroni corrections.
***Chi-square test was used to compare proportions between groups.
****Monte Carlo Exact test was used to compare proportions between groups.

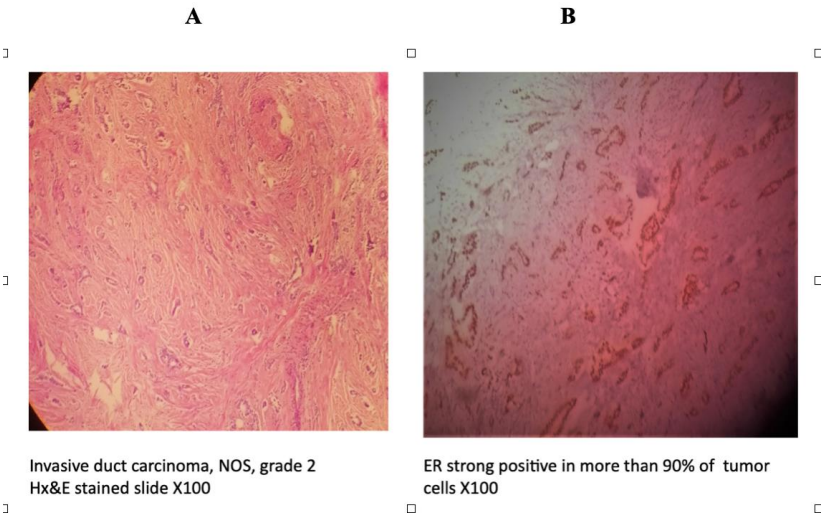

Figure 1: Microscopic picture by Hx&E (A) and ER staining (B)

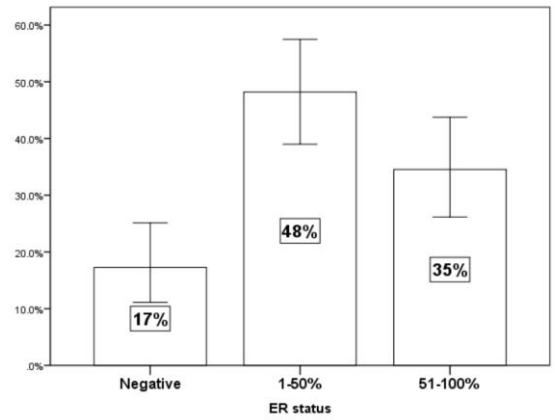

Figure 2: Levels of ER Expression in the study group

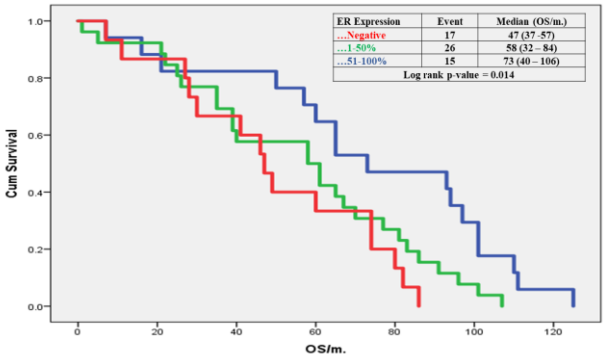

Figure 3: OS in negative, low/intermediate, and strong ER expression

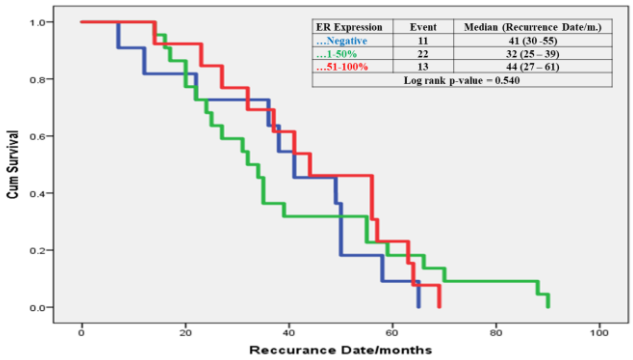

Figure 4: DFS in negative, low/intermediate, and strong ER expression

Discussion:

Our study correlates between the level of ER expression presented as a percentage of positively stained nuclei to breast cancer recurrence and survival. We compared the OS and DFS in three groups of patients according to the level of ER expression; negative, low/intermediate and strong (0%, 1-50% and 51-100%). The strong expression level to be > 50 % was proposed previously by experts of The St. Gallen international consensus on the primary therapy of early breast cancer 2009 [12].

There was a significant relation between the level of ER expression and T & N staging with p value = 0.025 and 0.048 respectively. This was also evident in PR expression with p value <0.001.

Regarding OS, our study showed a significant difference in the higher ER expression group than in the low/intermediate expression group (p value = 0.014).

Our results regarding the OS were more or less similar to a study done by Ryu et al , that recruited 4949 non metastatic breast cancer patients between 2003-2012 with 57.8 months median follow up period. In this retrospective study patients were divided into 3 groups; group I, group II and group III (ER negative, weak positive and strong positive) according to Allred total score. Ryu et al, found that the OS was significant in those with strong ER expression (group III) than in those with low ER expression (group II) (p value = 0.010) [13].

Our results were disconcordant with the study done by Zhang et al, which recruited 1700 patients during the period from 2000 to 2011 with a median follow up period of 5 years. This study divided the patients into 4 groups: (< 1%, 1-10%, 11-70% and >70%) and concluded that there was no significant difference in the OS in relation to level of ER expression (P = 0.2896) [14]. Also another study done by Morgan et al, which included 563 postmenopausal stage I and II breast cancer patients who received adjuvant hormonal treatment without chemotherapy. The patients divided according to positive ER stained cells into 3 groups (<34%, 34 to 67%, and >67%) and found that the 10 years OS was not significant in relation to the level of above ER expression (65.5%, 43.4%, and 70.9%, respectively) The discorcordance may be due to the greater sample size of these studies in relation to our study [15].

There was no significant difference in our study between levels of ER expression regarding DFS (p value= 0.540).

Our results regarding DFS were in concordance with the results of a study done by Campbell et al, which recruited 503 breast cancer patients during the period from 1995 till 1998 with 5.7 years of median follow up. This study found that there was no statistically significant difference between high and low ER expression in relation to DFS (p value= 0.21) [16].

On the opposite side our DFS results didn't match with the results of a study which recruited 4325 patients with 5 years median follow up period, and classified ER positive cases into ER rich (≤ 6) and ER poor (≥ 7) according to Allred score with statistically significant difference for ER rich group (p value= .002) [17]. Another study showed disconcordant DFS results done by Morgan et al; it showed that the 10 years DFS was highly significant in favor for the group with high ER expression (47.7%, 48.7%, and 76.3%, respectively, p value= 0.001) This difference may be due to longer

follow up and different eligibility criteria between compared studies [15].

There were some limitations in our study; it is a retrospective study with limited number of patients.

Conclusion:

Our study concluded that the level of ER expression had a significant impact on OS but this was not evident for DFS, there was also a direct relation between the level of ER expression and an early T&N staging of the tumor and positive PR expression.

Prospective multi-center study with large number of patients and correlation with other predictive and prognostic factors are required for confirmation of the results. Correlation with Her2 and Ki67 also needs to be defined in future studies.

List of abbreviations

HRs: Hormonal receptors; **ER**: Estrogen receptor; **Her2** : Human epidermal growth factor receptor 2; **OS**: Overall survival; **DFS**: disease free survival; **IHC:** Immunohistochemistry; **PR**: Progesterone receptor; **H(score)** : Histoscore; **SECI** : South Egypt Cancer Institute; **IRB**:Institutional Research Board; **IDC**: Invasive ductal carcinoma; **NST**: No specific type; **IBM-SPSS:** International Business Machines-Statistical Package for the Social Sciences; ; **HR:** Hazard ratio; **CI:** Confidence interval; **Hx&E**: Hematoxylin and eosin; **MRM** : Modified radical mastectomy; **BCS**: Breast conservative surgery.

Competing interests

The authors declare that they have no conflict of interests.

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