Cancer and Oncology Research 2(4): 51-57, 2014
DOI: 10.13189/cor.2014.020402 http://www.hrpub.org

Correlation of Breast Cancer Subtypes Based on ER, PR and HER2 Expression with Axillary Lymph Node Status

Elsayed M Ali[1,*], Ahmed R. H. Ahmed[2], Ayman M. A. Ali[3]

[1]Department of Clinical Oncology and nuclear medicine, Faculty of Medicine, Sohag University PO 82524 Egypt
[2]Department of Pathology Faculty of Medicine, Sohag University PO 82524 Egypt
[3]General Surgery Department, Sohag Faculty of Medicine, Egypt
*Corresponding Author: sayedmostafa07@hotmail.com

Abstract Background:Lymph node metastasis is the most important prognostic factor in breast cancer patients. The present study was carried out to evaluate the association between ER, PR and HER-2/neu expression status, separately and in combinations with axillary lymph node involvement. Patients and methods: Two hundred and fifty eight breast cancer patients treated with modified radical mastectomy with axillary lymph node dissection were included. The primary tumor tissue and axillary lymph node were evaluated histologically and the expression of ER, PR and HER-2/neu was evaluated by immune his to chemistry. Results: ER and PR expression were demonstrated in 78.7% and 76.4%, respectively and over-expression of HER-2/neu was detected in 13.2% of cases. Triple positive breast cancer is more likely to have axillary lymph node metastasis and ER+/PR+/HER-2- (PPN) is the most protected group ($p<=0.001$). There was a strong correlation between tumor size and tumor grade with lymph node involvement ($p= 0.0001$ and 0.024, respectively).
Conclusion: triple positive breast carcinomas are more likely axillary lymph node metastasis. Tumor size, tumor grade and pathological subtypes correlate with axillary lymph node status. Further confirmatory studies with a larger number of patients are necessary to define factors predicting axillary lymph node status.

Keywords Breast Cancer Subtypes, Axillary Lymph Node Status, Breast Carcinoma, ER, PR, HER-2

1. Introduction

Breast cancer is the most common malignant tumor among women. It accounts for 22% of all female cancers; more than twice the prevalence of cancer in women at any other site **[1]**. It has a varied spectrum of molecular, pathological and clinical features with different prognostic and therapeutic implications **[2]**.

The three predictive markers, estrogen receptor, progesterone receptor (ER, PR) and HER-2/neu have an independent prognostic value. ER expression was demonstrated in 80-90% of breast-cancer cases, while PR expression was demonstrated in 70-80% of cases **[3]**. HER-2/neuis over-expressed in about 15-20% of breast cancer cases **[4, 5]**.

Breast cancer is therefore, better represented by its combined receptor expression than by a single receptor status alone **[6, 7]**. Previous classification that separated breast cancer into one of two categories based on ER expression alone is less discriminatory in terms of prognosis, and the additional sub-classifications based on HER-2 expression enhanced more useful therapeutic guidance **[8]**.

The axillary lymph node (ALN) status is one of the best independent prognostic factors for disease-free survival and overall survival of breast cancer. The focus of many studies has been the identification of patients unlikely to benefit from ALN dissection, because the risk of nodal metastasis is low. The diagnostic use of sentinel lymph node biopsy could reduce the need of ALN dissection at the expense of increasing the risk of missed nodal metastasis **[9, 6]**. Rates of ALN involvement in different prognostic breast cancer subgroups based on their combined IHC (immune his to chemistry) expression of steroid receptors, and HER-2 status have not been systemically described.

2. Materials and Methods

2.1. Patients and Samples

Two hundred and fifty eight operable breast cancer patients were included within this study. All patients underwent radical excision with level I and II ALN clearance at Sohag University Hospital and Sohag Cancer Institute between January 2011 and June 2013. Approval for this work was obtained from Ethical Committee, Faculty of Medicine, Sohag University. A spreadsheet demonstrating the clinicopathological features of the patients, including age at diagnosis, menopausal status, tumor site, tumor size,

histological type and grade and lymph node status was constructed. Tumors are graded by a modification of Bloom and Richardson method **[10]** and the tumor size was the maximum tumor diameter.

2.2. Immunohistochemistry

Formalin-fixed paraffin-embedded tissue blocks of the primary tumor tissues were evaluated for the expression of ER, PR and HER-2/neu by IHC. Four micrometer-thick sections were deparaffinization xylene and rehydrated in down-regulated alcohols. The sections were washed in running water before incubation in 0.5% hydrogen peroxide for 10 minutes to block the endogenous peroxidase activity and then washed in running water. Antigen unmasking was by boiling in 10 mM citrate buffer, pH 6.0 in a microwave at high power for 2x5 minutes. Following antigen retrieval, the sections were left to cool down for 30 minutes and washed in Tris-buffered saline (TBS) pH 7.6. The tissues were incubated with the appropriate mouse monoclonal anti-human ER, PR or HER-2/neu primary antibody (Dako), diluted in TBS for one hour at room temperature. The sections were washed in TBS before incubation with peroxidase-labelled goat anti-mouse secondary antibody for 45 minutes at room temperature. The sections were washed with 0.5% TBS and exposed to 3,3′- diaminobenzidine tetrahydrochloride (DAB) solution to yield an insoluble brown deposit. Finally, the sections were counterstained with hematoxylin, dehydrated and mounted as usual. Replacement of the primary antibodies with TBS served as negative controls for the IHC process.

2.3. Immunohistochemistry Scoring

The expression of ER and PR was scored according to the scheme of Allred and his colleagues, 1998 **[11]**. Only nuclear immuno-reaction of the tumor invasive component was evaluated.

HER2/neuimmuno-reaction was scored according to the UK National External Quality Assessment Scheme for IHC **[12]**. This is a semi-quantitative method based upon the intensity of cell membrane immuno-reaction and the percentage of membrane positive cells, giving a score range of 0–3+. Score 0 is decided when no staining is observed or membrane staining in less than 10% of tumor cells, score 1+ when a faint partial membrane staining in more than 10% of tumor cells, score 2+ when a weak to moderate complete membrane staining in more than 10% of tumor cells and score 3+ when a strong complete membrane staining in more than 10% of tumor cells. Only score 3+ was considered as HER-2/neu over-expression while scores 0 and 1+ were considered negative. Score 2+ cases were evaluated with florescence in situ hybridization (FISH) to confirm Her2/neu over-expression.

2.4. Statistical Analysis

Based on immuno-reaction, our cases were classified into ER positive, P (score 2-8) or negative, N (score 0), PR positive, P (score 2-8) or negative, N (score 0) and HER2/neu over-expression, P (score 3+ and score 2+/positive FISH) or negative, N (scores 0, 1+ and score 2+/negative FISH). According to the combined expression of steroid and HER2/neu receptors, our cases can fall into one of eight possible categories: ER+, PR+ and HER2/neu+ (PPP), ER+, PR+ and HER2/neu- (PPN), ER+, PR- and HER2/neu+ (PNP), ER+, PR- and HER2/neu- (PNN), ER-, PR+ and HER2/neu+ (NPP), ER-, PR+ and HER2/neu- (NPN), ER-, PR- and HER2/neu+ (NNP) and ER-, PR- and HER2/neu- (NNN). The association between separate and combined expression of the three markers with lymph node status and with different clinicopathological parameters was evaluated statistically.

The commercially available statistical software (IBM-SPSS version 19.0 for Windows; IBM Inc) was used for data analysis. The frequency of a categorical observation among lymph node status was compared by Chi-Square Test (χ^2) and Fisher`s Exact Test. The correlation between lymph node status and other continuous variables was evaluated by Spearman`s rho Test. Pearson's correlation coefficient test was used to evaluate the association between continuous variables and Kruskal Wallis test was used to compare multiple variables.

3. Results

Two hundred and fifty eight patients were included within this study. The age of the patients ranged between 25-73 years with a mean (SD) of 50.4 (10.4) and a median of 51 years. One hundred and twenty-one of the patients (47%) aged 50 years or younger and 137 patients (53%) aged over 50 years. Thirty-four percent of the cases were pre-menopausal. 9% were peri-menopausal, and the remaining cases were post-menopausal. Tumor size ranged from 1 to 7cm with mean and median values of 3.6 cm and 3 cm, respectively. Ninety-two percent of the investigated tumors (n=239) was infiltrating duct carcinoma, not otherwise specified (IDC NOS) and 8% (n=19) were other types, including ten cases of medullary carcinoma, five cases of invasive lobular carcinoma, two cases of mixed duct lobular carcinoma and one case of each of tubular carcinoma and mucinous carcinoma. The majority of the investigated cases were grade II, representing 74.8% while grade, I and III occurred in 5.4% and 19.8%, respectively. Metastatic deposits of the tumor tissue in the ipsilateral ALN had been reported in 117 patients (45.3%). The number of ALN involved by metastatic deposits varied greatly among cases ranging from 1 to 43 lymph nodes with a median number of four.

Expression of both ER and PR was specifically nuclear while positive expression of HER2 was demonstrated as continuous membranous immunoreaction. The expression rates of ER and PR were 78.7% and 76.4%, respectively and

over-expression of HER2 (3+) was demonstrated in 34 cases (13.2%). One hundred and seventy of the investigated cases (65.9%) were ER positive; PR positive and HER2 negative (PPN). Simultaneous triple negative breast cancer (NNN) occurred in 15.5% (40 cases) and simultaneous triple positive breast cancer (PPP) occurred in 7.8% (20 cases). There was a strong association between ER and PR expression (χ^2 (1) =156.8, $p<0001$) while neither ER nor PR had a significant association with HER2 expression.

Among different clinical and pathological parameters of the studied breast cancer cases (Table 1), comparative analysis showed a strong correlation between tumor size with lymph node involvement (Spearman`s rho =0.638, $p<0.01$). Tumors with negative ALN have a significantly smaller average size compared to tumors with positive metastatic deposits within ALN. Nevertheless, tumor size is significantly associated with the increased number of involved ALN (Pearson`s correlation coefficient=0.513, $p<0.01$). In the same context, higher tumor grade is significantly associated with ALN metastasis, χ^2 (2) =49.7, $p<0.0001$ only 14.3% of grade I tumors had metastatic deposits compared to 36.3% of grade II tumors and 88.2% of grade III tumors. There was no association between lymph node status and patients` age, age groups or menopausal status. There is a significant higher rate of ALN involvement among invasive duct carcinoma NOS compared to other microscopic subtypes, χ^2 (2) =7.23, p=0.007, Fishers exact test, p=0.008.

Table 1. Correlation of clinical and pathological parameters with axillary lymph node status

Variable	Statistic	Lymph node status		All	p value*
		Negative	Positive		
Age (years)	Mean (SD) Median	50.16 (10.34) 51.00	50.65 (10.39) 51.00	50.38 (10.34) 51.00	0.69**
Age group <=50 years >50 years	 N (%) N (%)	 66 (25.6) 75 (29.1)	 55 (21.3) 62 (24.0)	 121(46.9) 137 (53.1)	0.537*
Menopausal status					
Pre-menopausal	N (%)	52 (20.2)	36 (14.0)	88 (34.1)	0.535*
Peri-menopausal	N (%)	13 (5.0)	10 (3.9)	23 (8.9)	
Post-menopausal	N (%)	76 (29.5)	71 (27.5)	147 (57.0)	
Pathology					
IDC	N (%)	125 (48.4)	114 (44.2)	239 (92.6)	0.007*
Other types	N (%)	16 (6.2)	3 (1.2)	19 (7.4)	
Tumor Size (cm)	Mean (SD) Median	2.7 (1.2) 2.5	4.7 (1.4) 5.00	3.6 (1.6) 3.00	0.0001**
Grade					
I	N (%)	12 (4.6)	2 (0.8)	14 (5.4)	0.0001*
II	N (%)	123 (47.7)	70 (27.1)	193 (74.8)	
III	N (%)	6 (2.3)	45 (17.5)	51 (19.8)	
ER					
Negative	N (%)	21 (8.1)	34 (13.2)	55 (21.3)	0.006*
Positive	N (%)	120 (46.5)	83 (32.2)	203 (78.7)	
PR					
Negative	N (%)	22 (8.5)	39 (15.1)	61 (23.6)	0.001*
Positive	N (%)	119 (46.1)	78 (30.3)	197 (76.4)	
HER2					
Negative	N (%)	133 (51.5)	91 (35.3)	224 (86.8)	0.0001*
Positive	N (%)	8 (3.1)	26 (10.1)	34 (13.2)	
ER/PR/HER2 subgroup					
PPP	N (%)	2 (0.8)	18 (6.9)	20 (7.7)	0.0001*
PPN	N (%)	113 (43.8)	57 (22.1)	170 (65.9)	
PNP	N (%)	2 (0.8)	1 (0.4)	3 (1.2)	
PNN	N (%)	3 (1.2)	7 (2.7)	10 (3.9)	
NPP	N (%)	1 (0.4)	2 (0.8)	3 (1.2)	
NPN	N (%)	3 (1.2)	1 (0.4)	4 (1.6)	
NNP	N (%)	3 (1.2)	5 (1.9)	8 (3.1)	
NNN	N (%)	14 (5.4)	26 (10.0)	40 (15.4)	

Chi square test* was used for categorical data and Spearman rho correlation coefficient test was used for quantitative data**

In separate analyses, the expression status of ER and PR showed inverse significant association with ALN metastasis. Only 83 of the 203 ER-positive breast cancers (41%) had positive ANL deposits compared to 34 of the 55 ER-negative tumors (62%); χ^2 (2) =7.65, p=0.006. Similarly, only 78 of the 197 PR-positive tumors (39.6%) had metastatic deposits in the ALN compared to 39 of the 61 PR-negative tumors (64%); χ^2 (2) =11.13, p=0.001. The four possible combinations of ER and PR status; ER+/PR+, ER+/PR-, ER-/PR+ and ER-/PR- were represented in 190, 13, 7 and 48 of the investigated cases; respectively. ER-/PR- tumors had a significant higher liability to send secondary deposits within ALN while ER+/PR+ tumors were the least likely to have metastatic deposits; χ^2 (3) =11.20, p<=0.011 (Figure 1A). Although ER+/PR- and ER-/PR+ breast cancer cases have no significant increased risk of ALN metastasis compared to ER+/PR+ cases; ER-/PR- cases have 2.8 times increase risk of metastasis in comparison to ER+/PR+ breast cancer cases (Binary logistic regression, p=0.002, 95% CI=1.45-5.41). This implies that expression of either ER or PR by the tumor could be a sign of good outcome for breast-cancer cases as regard ALN metastasis.

Over-expression of HER2 is significantly associated with presence of ALN deposits, χ^2 (2) =15.30, p<=0.001. Only 40.6% of the HER2 negative breast cancers had ALN metastasis compared to 76.5% of the HER2 positive cases (Figure 1B). Surprisingly, among the eight different combinations of ER/PR/HER2 expression status; the triple positive breast cancer (PPP) is more likely to have ALN metastasis and the ER+/PR+/HER2- (PPN) is the most protected group against metastasis, χ^2 (7) =36.70, p<=0.001 (Table 1). This finding reflects that HER2 over expression could be the most important defining factor for ALN metastasis among these three molecules. This was confirmed with the multivariate binary logistic regression analysis where HER2 expression had the strongest predictive value for ALN metastasis (Wald Chi square=12.38, p<0.0001), followed by PR expression (Wald Chi square=9.52, p<0.001) and lastly ER expression, which was insignificant. We also compared the risk of ALN metastasis among different ER/PR/HER2 combination subgroups in comparison to PPN subgroup, which was the least likely metastasizing breast cancer subgroup. None of the PNP, NPP, NPN or NNP showed significant increased risk of ALN metastasis compared to PPN breast cancer cases. In contrast, the triple positive and triple negative breast cancer have 17.8 and 3.7 times increase risk of ALN metastasis compared to PPN subgroups respectively and PNN breast cancer have 4.6 times increase risk of metastasis compared to PPN breast cancer (Table 2)

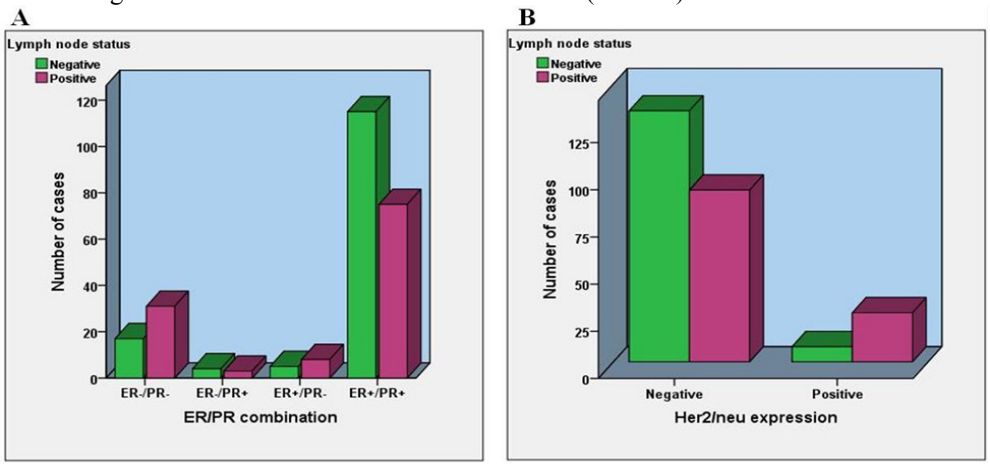

Figure 1. Correlation of ER/PR expression (A) and HER2 expression (B) with axillary lymph node status in breast cancer patients

Table 2. Probabilities of axillary lymph node metastasis in different combination subgroups of ER, PR and HER2 expression

ER/PR/HER2 subgroup	*P* value	Odds ratio	95% confidence interval	
			Lower	Upper
PPN		1		
PPP	*0.000*	17.842	4.000	79.577
PNP	0.994	0.991	0.088	11.164
PNN	*0.031*	4.626	1.153	18.562
NPP	0.265	3.965	0.352	44.655
NPN	0.722	0.661	0.067	6.496
NNP	0.110	3.304	0.762	14.318
NNN	*0.000*	3.682	1.786	7.591

PPN states for ER+/PR+/HER2-, PPP for ER+/PR+/HER2+, PNP for ER+/ PR-/HER2+, PNN for ER+/PR-/HER2-, NPP for ER-/PR+/HER2+, NPN for ER-/PR+/HER2-, NNP for ER-/PR-HER2+ and NNN for ER-/PR-/HER2-

The abovementioned results showed a strong association between ER and PR expression. Additionally, positivity of either ER or PR protects against ALN metastasis when compared to ER-/PR- cases. Based on these findings and for statistical purposes, the expression of both ER and PR were considered as one unit. The four possible combinations of this unit with HER2 expression status are either ER or PR+/HER2+ (PP), either ER or PR+/HER2- (PN), both ER.PR-/HER2+ (NP) and both ER.PR-/HER2- (NN). The number of cases in each of these subgroups was 26, 184, 8 and 40 cases for PP, PN, NP and NN, respectively. Again, ALN metastasis is more likely to occur in PP group and PN tumors are most likely to be protected from ALN deposits, χ^2 (3) =27.80, p<=0.001.PP and NN breast cancer cases have 7.7 times and 3.4 times increase risk of ALN metastasis compared to PN cases, respectively while NP cases had no significant rise for risk of metastasis (Table 3).

Table 3. Probabilities of axillary lymph node metastasis in different combination subgroups of ER.PR and HER2 expression

ER.PR/HER2 subgroup	P value	Odds ratio	95% confidence interval	
			Lower	Upper
PN		1		
PP	0.000	7.689	2.770	21.347
NP	0.135	3.051	.707	13.177
NN	0.001	3.400	1.661	6.961

PN states for either ER or PR+/HER2-, PP for either ER or PR+/HER2+, NP for both ER and PR-/HER2+ and NN for both ER and PR-/HER2-

4. Discussions

Prognosis of breast cancer is related to a large variety of clinical and pathological factors. ALN involvement represents an acceptable factor for predicting prognosis. Our study highlights the correlation of clinicopathological parameters and breast cancer subtypes with qualitative ALN involvement.

In our study, the mean age of all subjects was 50.4 years (SD, 10.4), and this finding was lower than that of a result reported by Onitilo, et al, 2009, who reported a mean age of 62.7 years (SD, 13.8). This result was near the result reported by Ayadi, et al, 2008 and this may require further careful examination to determine the nature of predisposing factors **[13, 14]**.

In this study, the most frequent histological subtype was invasive ductal carcinoma (92.6%), and included limited numbers of other subtypes.

In the present study, we found that, increasing tumor size and higher tumor grade are significantly associated with ALN involvement, and this result is in agreement with Patani, et al, 2007,Xie, et al, 2012 and Yoshihara et al, 2013 who reported that the tumor size and grade could have been the main reason for the higher probability of ALN involvement. The tumor size in our study is significantly associated with the increased number of involved lymph nodes (p< 0.01) **[15- 17]**.

In this study, there is a significant higher rate of ALN metastases among IDC patients compared to other microscopic subtypes (p= 0.007).

ER was positive in 78.7% and PR was positive in 76.4% PR of our investigated patients. It has been reported that 10-34% of breast carcinomas over-express HER2/neu receptor, in our study Over-expression of HER2/neu was13.2 percentage.

Tumor subtypes in our studies classified based on ER, PR expression and HER-2 over-expression, 10.1% of cases were ER/PR+, HER-2+ (PPP, PNP, NPP), 71.4% were ER/PR +, HER-2- (PPN, PNN, NPN), 3.1% were ER/PR-, HER-2+ (NNP), and 15.4% were classified as triple negative (NNN). The most common subtype in this study was ER/PR+, HER-2-, and this finding is in agreement with Onitilo, et al, 2009, results **[13]**.

Some studies reported no value for both ER and PR as a reliable predictor for ALN status **[9, 18].**Whereas other studies showed that, the presence of ER in a breast cancer is believed to coincide with low LN involvement risk **[19- 21]**. In our study, we found that ER-/PR- tumors had significant higher liability to send deposits in ALN, while ER+/PR+ tumors is the least likely to have ALN metastasis, also in a separate analysis, we found that, the expression of either ER or PR associated with low ALN involvement.

Almasriand Al Hamad, 2005, and Prati, et al, 2005 showed no statistically significant correlation between HER2/neu over-expression and ALN status. In the present study, overexpression of HER-2 is significantly associated with presence of ALN involvement and this result is in agreement with Huang, et al, 2005.**[22, 23]**.

In our study, among the eight combinations of ER/PR/HER-2, the triple positive breast cancer (PPP) is more likely to have ALN metastasis, and ER+/PR+/HER-2- (PPN) is the most protected group against ALN involvement. The previous finding is in agreement with Calster, et al, 2008 who reported that triple positive breast cancer had a higher potential for axillary lymph node involvement than breast cancers of any other ER/PR/HER2 phenotype **[24]**.

This finding reflects that HER-2 over expression could be the most important finding factor for ALN metastasis among these three molecules. We also found that, positivity of either ER or PR associated with the decrease incidence of ALN involvement, when compared to ER-/PR-. Based on the previous findings, the expression of both ER and PR should be considered as one unit, so according to this result the four possible combinations[ER or PR+/HER-2+ (PP), ER or PR+/HER-2- (PN), ER.PR+/HER-2+ (NP) and ER.PR-/HER-2- (NN)] should be used instead of the eight subtypes which based on PR expression.

Strength of our work is that cases were unselected and consecutively treated; weakness may be that we did not consider other predictors for ALN status as tumor localization within the breast, tumor vascularization, lymph angiogenesis, protein and genetic markers for ALN involvement.

5. Conclusions

Analysis of breast cancer subtypes and different clinicopathological parameters is important, because it provides valuable prognostic and predictive information. Our results showed that, triple positive breast carcinomas are more likely ALN positive than other subtypes. In addition, we found that, tumor size, tumor grade and pathological subtype correlate with lymph node status. The four subtypes are practical, simple, informative and quite discriminative between the subtypes. Further confirmatory studies are necessary to define factors to predict metastasis to axillary lymph node.

Abbreviations

ER: estrogen receptor; PR: progesterone receptor; HER2/neu: Herceptin receptor; ALN: axillary lymph node; IHC: immunohistochemistry.

Conflict of Interest

All authors have no conflict of interest

REFERENCES

[1] Lal P, Chen B. Correlation of HER-2 status with estrogen and progesterone receptors and histologic features in 3,655 invasive breast cancers. American Journal of Clinical Pathology, 123 (4): 541-6, 2005.

[2] Carey L A, Perou C M, Livasy C A,et al. Race breast cancer subtypes and survival in the Carolina breast cancer study. JAMA, 295: 2492- 502, 2006.

[3] Grann VR, Troxel AB, ZojwallaNJ,JacobsonJS,Hershman D, Neugut AI. Hormone receptor status and survival in a population- based cohort of patients with breast carcinoma. Cancer, 103(11): 2241- 51, 2005

[4] Esteva FJ, Hortobagyi GN. Prognostic molecular markers in early breast cancer. Breast Cancer Res. 2004; 6: 109- 18, 2004.

[5] Ross JS, Fletcher JA,LinetteGP.The HER-2 gene and protein in breast cancer: biomarker and target of therapy. Rev Oncologist, 8: 307- 25, 2003.

[6] SorlieT, Perou CM, Tibshirani R, et al. Gene expression patterns of breast cancers distinguish tumor subclasses with clinical implications. ProcNatlAcadSci USA, 98(19): 10869- 74, 2001.

[7] Yao ZX, Lu LJ, Wang RJ, et al. Discordance and clinical significance of ER, PR and HER2 status between primary breast cancer and synchronous axillary lymph node metastasis. Med Oncol, 31(1): 798, 2010.

[8] Carey L A, Dees E C, Sawyer L,et al. The triple negative paradox: primary tumor chemosensitivity of breast cancer subtypes. Clin Cancer Res, 13: 2329- 34, 2007.

[9] Gagdos C, Tartter P I, Bleiweiss I J. Lymphatic invasion, tumor size, and age are independent predictors of axillary lymph node metastasis in women with T1 breast cancers. Ann Surg, 230 (5): 692, 1999.

[10] Elston CW and Ellis IO: Assessment of histological grade. In Elston, C.W. and Ellis, I.O. (eds), The breast. Vol. 13. Churchill Livingstone, Edinburgh, New York pp 356-84, 1998.

[11] Allred D C, Harvey JM, BerardoM,ClarkGM. Prognostic and predictive factors in breast cancer by immunohistochemical analysis. Mod Pathol, 11(2): 155-68, 1998.

[12] Ellis IO: Pathology Reporting of Breast Disease. A Joint Document Incorporating the Third Edition of the NHS Breast Screening Programme's Guidelines for Pathology Reporting in Breast Cancer Screening and the Second Edition of the Royal College of Pathologists' Minimum Dataset for Breast Cancer Histopathology. 2ndedn. NHS Cancer Screening Programmes& Royal College of Pathologists. Sheffield: NHS Cancer Screening Programmes&The Royal College of Pathologists 2005.

[13] Onitilo A A, Engel J M, Greenlee R T,Mukesh BN: Breast cancer subtypes based on ER/PR and HER-2 expression: comparison of clinicopathologic features and survival. Clinical Medicine & Research, 7: 4- 13, 2009.

[14] Ayadi L, Khabir A, AmouriH,et al. Correlation of HER-2 over-expression with clinic-pathological parameters in Tunisian breast carcinoma. World Journal of Surgical Oncology 2008; 6: 112.

[15] Patani N R, Dwek M V, Douek M: Predictors of axillary lymph node metastasis in breast cancer: a systemic review. Eur J SurgOncol, 33: 409- 19, 2007.

[16] Xie F, Yang H, Wang S,Zhou B, Yang D, Zhang J. A logistic regression model for predicting axillary lymph node metastases in early breast carcinoma patients. Sensors (basal), 12 (7): 9936- 50, 2012.

[17] Yoshihara E, Smeets A, Laenen A, et al. Predictors of axillary lymph node metastases in early breast cancer and their applicability in clinical practice. The Breast, 22: 357-61, 2013.

[18] Chua B, Ung O, Taylor. Frequency and predictors of axillary lymph node metastasis in invasive breast cancer. ANZ J Surg, 71: 723- 28, 2001.

[19] Nouh M A, Ismail H, El-Din N H,El-BolkainyMN.Lymph node metastasis in breast cancer: clinicopathological correlation in 3747 patients. J. Egypt. Natl. Cancer Inst, 16: 50-6, 2004.

[20] Cetintas S K, Kurt M, OzkanL,Engin K, Gokgoz S, Tasdelen I. Factors influencing axillary lymph node metastasis in breast cancer. Tumori, 92: 416- 22, 2002.

[21] Bauer K, Parise C, Caggiano V. Use of ER/PR/HER2 subtypes in conjunction with the 2007 St Gallen consensus statement for early breast cancer. BMC Cancer, 10: 228, 2010.

[22] Almasri N M, Al HamadM.Immunohistochemical evaluation of human epidermal growth factor receptor 2 and estrogen and progesterone receptors in breast carcinoma in Jordan.

Breast Cancer Res, 7: 598- 604, 2005.

[23] PratiR, Apple S K, He J,Gorbein J A, Chan H R.Histopathologic characteristics predicting HER-2/neu amplification in breast cancer. Breast J, 11(6): 433- 39, 2005.

[24] Calster BV, BemptIV, DrijkaningenM,et al. Axillary lymph node status of operable breast cancer by combined steroid receptors and HER-2 status: triple positive tumors are more likely lymph node positive. Breast Cancer Res Treat, DOI: 10.1007/5 10549-008-9914-7, 2008.