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HER2 expression is a strong independent predictor of nodal metastasis in breast cancer

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KEYWORDS

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Abstract Identification of metastatic potential of breast cancer cells is necessary for proper management of this disease. This work aimed to estimate likelihood of axillary lymph node (ALN) involvement in breast cancer patients based on human epidermal growth factor receptor 2 (HER2) expression. Primary tumors of 317 breast cancer patients were evaluated for estrogen receptor (ER), progesterone receptor (PR) and HER2 expression by immunohistochemistry. The validity of these molecules to predict ALN metastasis was measured statistically and compared to predictive effect of other clinicopathological parameters. ER, PR and HER2 expression was detected in 75.7%, 73.2% and 19.9% of tumors, respectively. Although increased tumor size and grade, ER and PR negativity and HER2 positivity were strong indicators of ALN metastasis on univariate analyses, only tumor size and HER2 expression were independent predictors of ALN involvement on multivariate analysis. ROC curve showed a strong validity of the model using these two parameters to predict ALN status (AUC 0.86; $p < 0001$). HER2-rich, luminal B and triple negative tumors had 6.87, 6.32 and 3.58 times increased risk of metastasis compared to luminal A tumors; respectively. HER2 expression in pT1 and pT2 tumors raised the risk of ALN metastasis by 7.7 and 7.6 times, respectively and grade 1 and 2 tumors that expressed HER2 were 16.0 and 7.8 times more likely to have ALN metastasis, respectively. To conclude, HER2 expression is associated with a significant rise of metastatic potential of breast cancer cells and could be a strong indicator of regional and distant metastasis of breast cancer.

Introduction

Breast cancer is still the most frequent malignant tumor in women worldwide with nearly 1.7 million new cases diagnosed in 2012, accounting for 25% of all new female cancer cases [1]. In Egypt, the rate of breast cancer is higher than the worldwide records representing 32.04% of female cancers diagnosed during 2008–2011 [2]. More importantly; it has been reported that 49.7% of the Egyptian patients have regional spread at the

Abbreviations: ALN, axillary lymph node; ER, estrogen receptor; PR, progesterone receptor; HER2, human epidermal growth factor receptor 2; ROC, receiver operating characteristic; pT, pathological stage

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time of diagnosis and 11.9% of them have distant metastasis [3].

Breast cancer is a heterogeneous disease with different molecular subtypes, cellular composition, clinical behavior and response to treatment. Hence, pathological features such as tumor size, tumor grade, nodal involvement and hormone receptor status are essential for management and prognosis of this disease. Axillary lymph node (ALN) status is the best independent prognostic factor and is essential for treatment planning of breast cancer patients. Selection of type and intensity of neo-adjuvant and adjuvant chemotherapy of breast cancer is based mainly on ALN status and number of involved lymph nodes [4]. Excision of the primary tumor with ALN dissection has been the standard surgical treatment of breast cancer. However, dissection of ALN is commonly associated with local complication of the related upper limb including lymphedema, impaired shoulder movement, pain and numbness [5]. The need for ALN dissection has been constantly reduced because of the introduction of sentinel lymph node biopsy. However; sentinel lymph node biopsy is still an invasive procedure and could be associated with a risk of missed nodal involvement and low sensitivity particularly in cases with micro-metastasis [6].

Several studies have been conducted to predict ALN metastasis in order to select patients unlikely to get benefit from ALN dissection. Size, grade and micro-vessel density of the primary tumors were stated to be independent predictors to signify ALN metastasis [7,8]. In addition, several molecules including estrogen receptor (ER), progesterone receptor (PR) and Ki-67 had been shown to have a predictive validity of nodal metastasis of breast cancer [9]. Among molecules that raised much attention as a possible prognostic marker is epidermal growth factor receptor 2; also known as HER2/neu or c-erbB-2. HER2 is a proto-oncogene that encodes a transmembrane receptor with a constitutive tyrosine kinase activity involved in cellular proliferation, differentiation, migration, and apoptosis. It is overexpressed due to gene amplification in 15–30% of human breast cancers [10]. In his review, Ross et al. [11] reported that HER2 amplification or overexpression was associated with poor outcome in patients with ALN metastases, but not in patients with tumor-negative lymph nodes. In this study, the validity of HER2 to predict ALN metastasis of breast cancer was evaluated and compared to other established predictive clinical and pathological parameters.

Materials and methods

Patients and samples

Ethical permission for this study was obtained from Ethics Committee, Faculty of Medicine, Sohag University. All breast cancer cases treated surgically at Sohag Cancer Institute or Sohag University Hospital during January 2014 to December 2015 were included in this study. The main inclusion criterion was surgical treatment by either radical mastectomy or conservative breast surgery with complete ALN dissection. Patients were excluded from the study if the primary tumor was previously excised, if the tumor was locally advanced (stage 4), if the patient underwent only ALN sampling, if they had distant metastasis at time of diagnosis and if they received pre-operative neo-adjuvant therapy. Formalin-fixed paraffin-embedded tissue blocks of 317 tumors were retrieved for the study. The patients were treated with modified radical mastectomy ($n = 207$) or conservative breast surgery ($n = 110$) with complete dissection of ALNs. Clinical data were obtained from the patient's clinical files and sections from primary tumors and ALNs were reviewed for evaluation of histopathological findings. The tumors were graded according to the Bloom and Richardson scheme [12] and the tumor size was the maximum tumor diameter. The pathological stage of the primary tumor was classified according to maximum diameter into pT1 for tumors up to 2 cm, pT2 for tumors of 2.1–5 cm and pT3 for tumors more than 5 cm [13].

Immunohistochemistry

Four micrometer-thick sections were de-deparaffinized in xylene and rehydrated in down-graded alcohols before washing thoroughly in running water. The sections were incubated in dual endogenous enzyme blocking solution (Dako Code K4065) for 10 min to block endogenous peroxidase activity followed by washing in running water. Antigen unmasking was by boiling the sections in 10 mM citrate buffer, pH 6.0 in a microwave at high power for 2×5 min; followed by cooling down to room temperature for 30 min. The sections were washed in phosphate-buffered saline (PBS) pH 7.6 before incubation with either mouse monoclonal anti-human ER-α (Dako, clone 1D5, code M7047), mouse monoclonal anti-human PR (Dako, clone 636, code M3569) or rabbit polyclonal anti-human c-erbB-2 protein (Dako, code A0485) for 30 min at room temperature. After washing twice in PBS, the sections were incubated with Peroxidase labeled polymer conjugated to goat anti-mouse and goat anti-rabbit immunoglobulin (Dako, code K4065) for 30 min at room temperature. The sections were washed with 0.5% PBS and exposed to a freshly prepared 3,3′-diaminobenzidine tetrahydrochloride (DAB) solution (Dako, code K4065) for 5–10 min 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.

Immunohistochemistry scoring and intrinsic subtyping of breast cancer

The expression of ER and PR was scored according to Allred scheme which is based on summation of proportion of positive cells and staining intensity [14]. Only nuclear immunoreaction of the invasive tumor component was evaluated and the final score was 0 or 2–8. Allred score 0 or 2 were classified as hormone receptor negative while Allred score 3–8 were considered as hormone receptor positive and this is concordant to recommendations of the American society of clinical oncology/College of American Pathologists (ASCO/CAP) that consider expression of hormone receptors to be negative if less than 1% of tumor cells was immunoreactive [15]. HER2 immunoreaction was evaluated in only invasive tumor cells and scored according to the updated ASCO/CAP recommendations [16]. This is a semi-quantitative scoring system based on intensity of cell membrane immunoreaction and the percentage of membrane positive cells, giving a score range of 0–3+. Only score 3

+ tumors were classified as HER2-positive while scores 0 and 1+ tumors were considered as HER2-negative. Equivocal cases (Score 2+) were excluded from the study as confirmation of HER2 gene amplification with florescence in situ hybridization (FISH) was not available in the institution. According to expression status of ER, PR and HER2 molecules; tumors included in this study were categorized into four groups: luminal A for ER+/PR+/HER2− tumors, luminal B for ER+/PR−/HER2−, ER−/PR+/HER2− and ER and/or PR+/HER2+ tumors, HER2-enriched (non-luminal) for ER−/PR−/HER2+ tumors and triple negative for ER-/PR-/HER2- tumors [17].

Statistical analysis

The commercially available statistical software IBM-SPSS (version 22 for windows; IBM Inc.) was used for data analysis. A p-value of less than 0.05 was considered as statistically significant. Chi-square test (χ^2) was performed to compare the rates of ALN metastasis between different study groups. The association of two continuous variables was analyzed by Spearman's rho test and the association between continuous and grouped variables were analyzed by either Mann–Whitney U or Kruskal–Wallis test. Univariate binary logistic regression analysis was performed to assess risk factors for ALN metastasis and to calculate odds ratio and 95% confidence intervals (CI) for probabilities of ALN metastasis. Variables with p-values <0.05 in univariate analyses were included in a multivariate regression analysis using a foreword selection procedure to identify the independent risk factors of ALN metastasis and to select the best predictive model of nodal metastasis. The receiver operating characteristic (ROC) curve was constructed and the area under the curve (AUC) was used to assess the predictive accuracy of the model.

Results

Three hundred and seventeen women with breast cancer were included in this study. The age ranged between 24 and 77 years with a mean (±SD) of 50.32 (±10.67) years and a median of 51.00 years. Nearly half of the investigated cases (48.27%) were aged 50 years or younger and more than one fourth of them (25.56%) were pre-menopausal. The tumor size ranged between 1.00 and 11.00 cm with mean (±SD) and median values of 3.72 (±1.75) cm and 3.00 cm, respectively. Based on their sizes, the tumors were classified as pT1, pT2 and pT3 in 80, 187 and 50 cases, respectively. Ninety percent of the investigated tumors (n = 284) were invasive duct carcinoma, not otherwise specified (IDC NOS) while invasive lobular, medullary, papillary, mucinous, tubular, and cribriform subtypes were recorded in 13, 12, 3, 2, 2, and 1 cases, respectively. The majority of tumors were grade II, representing 74.1% while grade I and III occurred in 6.3% and 19.6%, respectively. ALN metastasis was confirmed histologically in nearly half of the investigated cases (n = 157) and the number of involved lymph nodes ranged between 1 and 32 with a median number of 6.

Expression of ER and PR was detected in 240 (75.7%) and 232 (73.2%) tumors, respectively and overexpression of HER2 protein (score 3+) was evident in 63 (19.9%) of the investigated cases. Diffuse strong expression of ER and PR (Allred score 8) was recorded in 181 and 173 cases, respectively. The expression of ER and PR was specifically nuclear (Fig. 1A and B) while expression of HER2 was demonstrated as a continuous membranous immunoreaction (Fig. 1C). The tumor stroma showed always negative expression of the three molecules. Based on combined expression of ER, PR and HER2 molecules, 192 of the tumors were classified as luminal type A while luminal type B, HER2-rich and triple negative tumors were recorded in 56, 23 and 46 cases, respectively. Among the luminal B tumors; 16 were luminal/HER2-negative and 40 were luminal/HER2-positive cases.

Among different investigated parameters of breast cancer (Table 1); increase of tumor size and grade and positive expression of HER2 were strongly associated with ALN metastasis. Alternatively; positive expression of both ER and PR seems to reduce the probability of ALN involvement. Nonetheless, the number of involved lymph nodes was significantly higher in large sized tumor (r = 0.575; $p < 0.0001$), in high grade tumors (Kruskal–Wallis test, $p < 0.0001$), in HER2-positive tumors (Mann–Whitney U test, $p < 0.0001$), in ER-negative tumors (Mann–Whitney U test, $p = 0.008$) and in PR-negative tumors (Mann–Whitney U test, $p < 0.001$). Tipple negative, HER2-rich and luminal B breast cancer had significantly higher rates of ALN metastasis [χ^2 (3) = 46.4, $p < 0.0001$].

According to combined expression of steroid and HER2 receptors, breast cancer would fall into one of eight possible categories: ER+/PR+/HER2+, ER+/PR+/HER2−, ER+/PR−/HER2+, ER+/PR−/HER2−, ER−/PR+/HER2+, ER−/PR+/HER2−, ER-/PR-/HER2+ and ER−/PR−/HER2−. These eight categories were presented in this study in 33, 192, 3, 12, 4, 4, 23 and 46 cases, respectively. Among these categories; the triple positive and ER−/PR−/HER2+ breast cancer subtypes had frequent ALN metastasis while ER+/PR+/HER2− breast cancer was the least likely to metastasize (Table 2). In the same context, luminal B and HER2-rich breast cancer; both of which showed frequent overexpression of HER2 had 6.32 and 6.87 times increase risk of ALN metastasis; respectively compared to luminal A breast cancer which is HER2 negative while triple negative tumors had only 3.58 times increased risk of metastasis (Table 3). These findings reflect that HER2 overexpression could be the most important defining factor for ALN metastasis among the three molecules.

The validity of HER2 expression to predict ALN metastasis was compared to other pathological parameters of breast cancer. Although increased tumor size and grade, ER and PR negativity and HER2 positivity were strong indicators of ALN metastasis on univariate regression analyses, only tumor size and HER2 expression were independent strong predictors of ALN involvement on multivariate regression analysis (Table 4). ROC curve showed a strong validity of the model using these two parameters in predicting the status of ALN (AUC 0.86; SE 0.021; CI 0.82–0.90, $p < 0001$, Fig. 2). This percentage was not improved when tumor grade was included in the predictive model.

To neutralize the effect of tumor size and grade, the associations of HER2 expression with ALN metastasis in pT1, pT2, grade 1 and grade 2 tumors were separately evaluated. There were significant associations of HER2 expression and ALN metastasis in early stages and low grades breast cancer (Fig. 3). Among pT1 tumors, HER2-positivity was associated

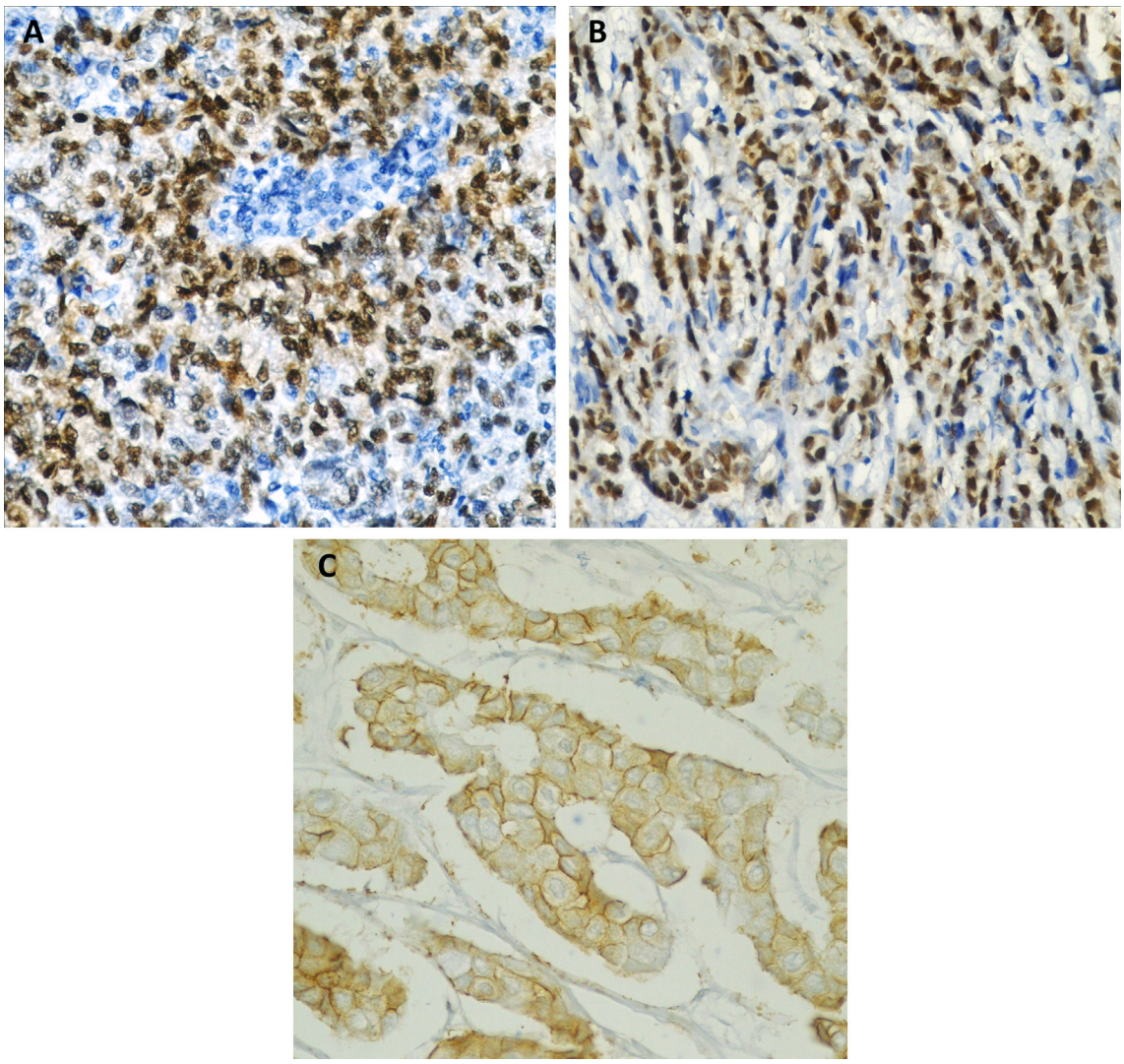

Figure 1 Nuclear expression of ER (A) and PR (B) and membranous expression of HER2 (C) in breast cancer. Magnification is 400× for A, B and C.

with 7.7 times higher risk of ALN metastasis (95% CI = 1.8–32.7, $p = 0.005$) and among pT2 tumors, HER2 expression raised risk of ALN metastasis by 6.7 times (95% CI = 2.5–18.1, $p < 0.0001$). Similarly; grade 1 tumors that expressed HER2 were at least 1.2 times more likely to have ALN metastasis (95% CI = 1.2–200, $p = 0.032$) and grade 2 tumors that expressed HER2 were 7.8 times more likely to have nodal metastasis (95% CI = 3.4517.0, $p < 0.0001$).

Discussion

Breast cancer is the main leading cause of cancer deaths among Egyptian women and worldwide [2,3]. It is not the primary tumor; but metastases that are the main cause of death in these patients. ALN involvement is the main route for spread of breast carcinoma. Pre-operative identification of patients with high risk of ALN metastasis would enable surgeons and oncologists to plan treatment strategies to individual patients. In a study that included 3747 cases of breast cancer in Egypt; Nouh et al. [8] demonstrated that tumor size and old age significantly raised the likelihood of ALN metastasis. In this study; the validity of ER, PR and HER2 expression to predict metastatic potential of breast cancer in Egyptian women was evaluated and compared to other clinical and pathological parameters. Categorization of breast cancer according to ER, PR and HER2 expression was also provided.

Fifty-one of the investigated cases (16.1%) had pT3 tumor stage and ALN metastasis was detected in 49.5% of which 36.3% and 29.3% were classified as pN2 and pN3 nodal stage, respectively. Weak screening programs, non-systematic health insurance and cultural and socioeconomic considerations could be explanatory reasons for the late stage at diagnosis of breast cancer among Egyptian women. The rates of ER, PR and HER2 expression in this study and the rates of intrinsic subtypes of breast cancer based on expression of these three molecules are comparable to records of previous literature [13,18,19].

According to this study; increased tumor size, high tumor grade, negative expression of hormone receptors and positive expression of HER2 were strongly associated with ALN

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

Variable	Statistic	Lymph node status		All	*p* value
		Negative $N = 160$	Positive $N = 157$		
Age (years)	Mean (SD)	50.59 (10.77)	50.04 (10.60)	50.32 (10.67)	0.713**
	Median	52.00	50.00	51.00	
Menopausal status					
Pre-menopausal	*N* (%)	44 (27.5)	37 (23.6)	81 (25.6)	0.609*
Peri-menopausal	*N* (%)	28 (17.5)	33 (21.0)	61 (19.2)	
Post-menopausal	*N* (%)	88 (55.0)	87 (55.4)	175 (55.2)	
Pathology					
IDC NOS	*N* (%)	140 (87.5)	144 (91.7)	284 (89.6)	0.219*
Other types	*N* (%)	20 (12.5)	13 (8.3)	33 (10.4)	
Tumor size (cm)	Mean (SD)	2.82 (1.29)	4.64 (1.67)	3.72 (1.75)	0.0001**
	Median	3	4.50	3.00	
pT stage					
pT1	*N* (%)	67 (41.9)	13 (8.3)	80 (25.2)	
pT2	*N* (%)	87 (54.3)	100 (63.7)	187 (59.0)	0.0001*
pT3	*N* (%)	6 (3.8)	44 (28.0)	50 (15.8)	
Grade					
I	*N* (%)	13 (8.1)	7 (4.5)	20 (6.3)	0.0001*
II	*N* (%)	136 (85.0)	99 (63.0)	235 (74.1)	
III	*N* (%)	11 (6.9)	51 (32.5)	62 (19.6)	
ER					
Negative	*N* (%)	25 (15.6)	52 (33.1)	77 (24.3)	0.0001*
Positive	*N* (%)	135 (84.4)	105 (66.9)	240 (75.7)	
PR					
Negative	*N* (%)	27 (16.9)	58 (36.9)	85 (26.8)	0.0001*
Positive	*N* (%)	133 (83.1)	99 (63.1)	232 (73.2)	
HER2					
Negative	*N* (%)	149 (93.1)	105 (66.9)	254 (80.1)	0.0001*
Positive	*N* (%)	11 (6.9)	52 (33.1)	63 (19.9)	
Molecular subtype					
Luminal A	*N* (%)	126 (78.8)	66 (42.0)	192 (60.5)	0.0001*
Luminal B	*N* (%)	13 (8.1)	43 (27.4)	56 (17.7)	
HER2-rich	*N* (%)	5 (3.1)	18 (11.5)	23 (7.3)	
Triple negative	*N* (%)	16 (10.0)	30 (19.1)	46 (14.5)	

Chi square test* was used for categorical variables and Spearman rho correlation coefficient test was used for quantitative variables**. IDC NOS states for invasive duct carcinoma, not otherwise specified, pT for pathological stage, ER for estrogen receptor, PR for progesterone receptor and HER2 refers to human epidermal growth factor receptor 2.

Table 2 Risk of axillary lymph node metastasis in breast cancer subtypes defined by ER, PR and HER2 expression.

Breast cancer subtype	Total number	Number (%) of involved axillary lymph nodes	*p* value	Odds ratio	95% Confidence interval	
					Lower	Upper
ER+/PR+/HER2−	192	66 (34.4%)		Reference		
ER+/PR+/HER2+	33	30 (90.9%)	0.000	19.091	5.616	64.898
ER+/PR−/HER2−	12	8 (66.7%)	0.034	3.818	1.109	13.150
ER−/PR+/HER2−	4	1 (25.0%)	0.698	.636	.065	6.238
ER+/PR−/HER2+	3	1 (33.3%)	0.970	.955	.085	10.722
ER−/PR+/HER2+	4	3 (75.0%)	0.134	5.727	.584	56.144
ER−/PR−/HER2+	23	18 (78.3%)	0.000	6.873	2.442	19.340
ER−/PR−/HER2-	46	30 (65.5%)	0.000	3.580	1.821	7.037

Table 3 Risk of axillary lymph node metastasis among different intrinsic breast cancer subtypes:

Breast cancer subtype	Total number	Number (%) of involved axillary lymph nodes	*p* value	Odds ratio	95% Confidence interval	
					Lower	Upper
Luminal A	192	66 (34.4%)		Reference		
Luminal B	56	43 (76.8%)	.000	6.32	3.17	12.57
HER2-rich	23	18 (78.3%)	.000	6.87	2.44	19.34
Triple negative	46	30 (65.2%)	.000	3.58	1.82	7.04

Table 4 Univariate and multivariate regression analysis of factors associated with axillary lymph node metastasis.

Parameter	Univariate regression co-efficient (95% CI)	*p* value	Multivariate regression co-efficient (95% CI)	*p* value
Increased tumor size	2.568 (2.057–3.207)	0.000	2.351 (1.852–2.984)	0.000
Increase tumor grade				
Grade II	2.038 (0.711–5.845)	0.185	1.194 (0.341–4.179)	0.781
Grade III	14.84 (4.362–50–49)	0.000	6.295 (1.473–26.91)	0.013
Negative ER expression	2.674 (1.557–4.593)	0.000	0.935 (0.306–2.854)	0.805
Negative PR expression	2.886 (1.706–4.881)	0.000	1.545 (0.520–4.592)	0.434
Positive HER2 expression	6.708 (3.342–13.46)	0.000	5.503 (2.433–12.45)	0.000

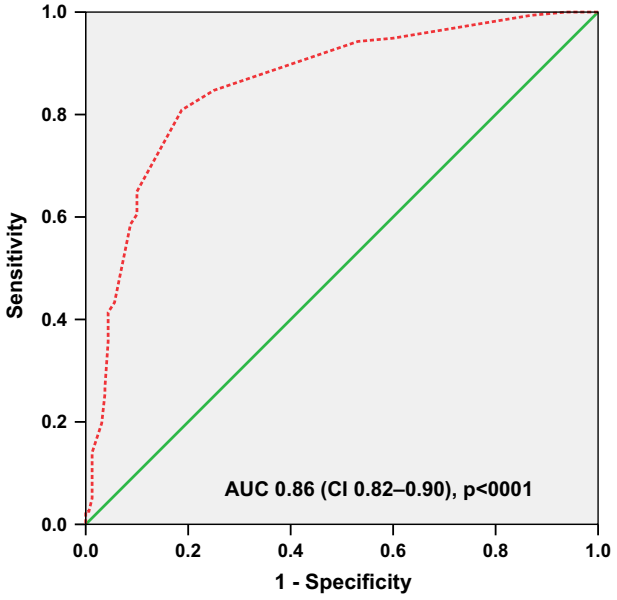

Figure 2 ROC curve for predicting axillary lymph node metastasis of breast cancer using tumor size and status of HER2 expression.

metastasis. There is a consensus that tumor size, grade and lymphovascular invasion are strong predictors of ALN metastasis, disease free survival and overall survival of breast cancer [20]. Although ER and PR are well established strong predictors of hormonal therapy of breast cancer patients; their validity to predict ALN metastasis is controversial. Few reports referred to a negative association of hormone receptor expression and ALN metastasis [21] while others reported that expression of ER and PR was associated with a relative increased risk of metastasis [22]. Still other studies showed no relationship of ER or PR to ALN metastasis [23,24]. In this study, the expression of both ER and PR was predictive of absent nodal metastasis on univariate but not multivariate models which suggests a weak validity of both molecules in comparison to other parameters.

HER2 expression was a strong predictive factor of ALN metastasis on univariate regression analysis and it was the second strongest factor after tumor size in multivariate analysis (Table 4). In the same context, HER2-rich breast cancer subtype had the highest risk of ALN metastasis compared to luminal A tumors (Table 3) followed by luminal B subtype which included a considerable number of HER2-positive cases. Among luminal B cases; HER2-positive cases showed a significant association with ALN metastasis compared to HER2-negative cases (Chi-square = 5.29, $p = 0.021$). Moreover; HER2 expression was strongly associated with ALN metastasis in early stages and low grades breast cancer cases (Fig. 3). Taken together; these data imply that whenever expressed, HER2 is associated with a significant higher risk of ALN metastasis of breast cancer regardless of tumor size, grade, hormonal status or intrinsic subtype. In separate large studies; luminal/HER2 and HER2-enriched intrinsic subtypes had higher tumor and nodal stage, frequent regional recurrence, early relapse rates and worse overall survival compared to other subtypes [25,26]. In the same context, several studies showed that triple negative breast cancer which is a distinctive subtype with aggressive clinical course and poor outcome had significantly lower rates of ALN positivity compared to HER2-positive breast cancer subtypes [9,27,28]. Among the eight possible combinations of ER, PR and HER2 expression status; the triple positive and ER−/PR−/HER2+ breast cancer subtypes had 19.1 and 6.9 times increased risk of ALN metastasis; respectively compared to ER+/PR+/HER2− tumors which was the most frequent subtype in this study and the least likely to have ALN metastasis. This is concordant

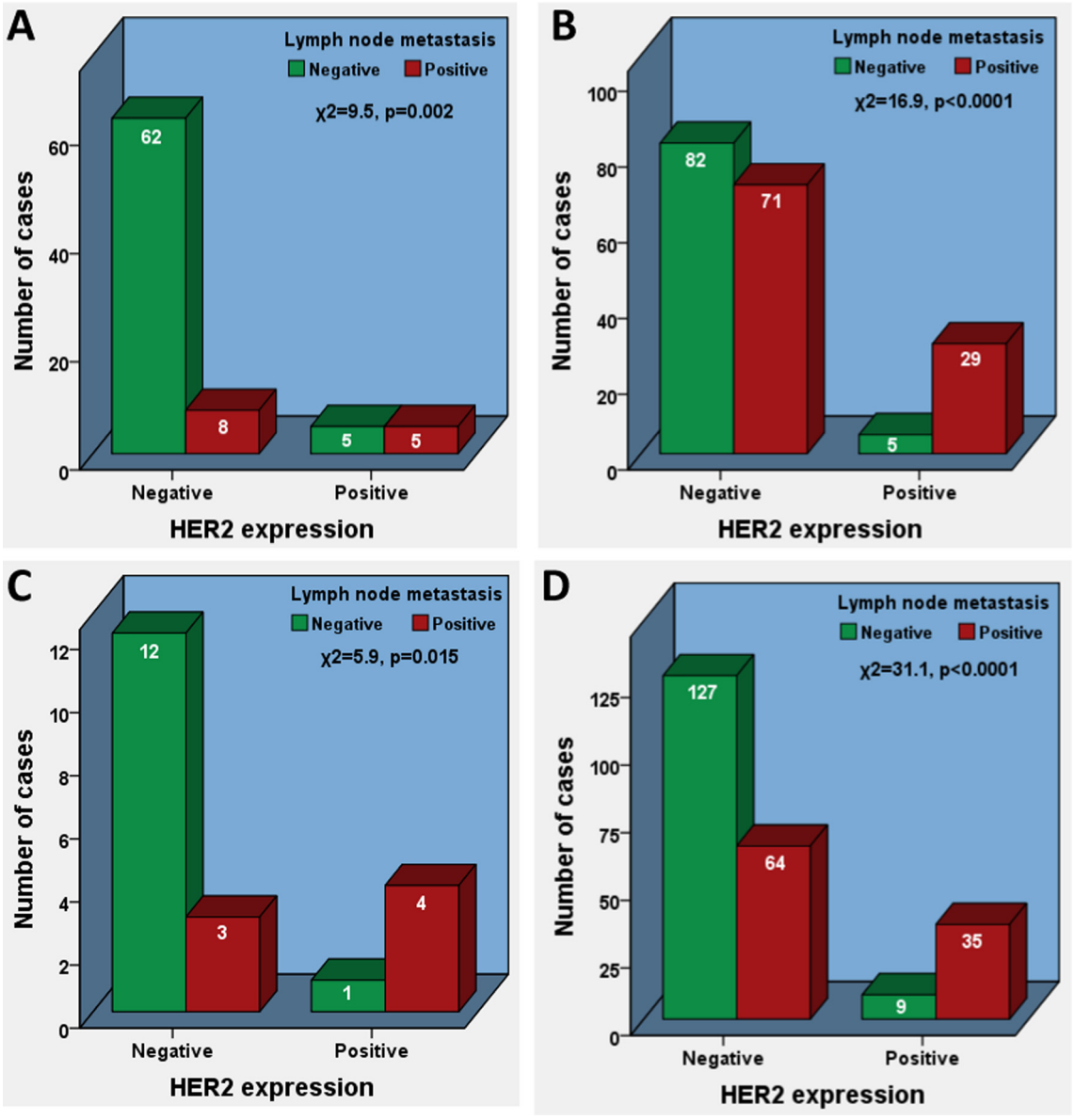

Figure 3 Association of HER2 expression with axillary lymph node metastasis in pT1 (A), pT2 (B), grade 1 (C) and grade 2 (D) breast cancer.

with previous literature [21,22] and confirms that HER2 overexpression is an important driver for breast cancer cells to metastasize. Accordingly, expression status of HER2 could be valuable to predict metastatic potential of breast cancer cells. Multivariate regression analysis of different clinical and pathological characteristics of breast cancer showed that combination of tumor size and HER2 expression status had the best performance to predict ALN metastasis. The model using these two parameters showed a strong predictive validity of ALN metastasis on ROC curve analysis with AUC of 86% (CI: 82–90%, $p < 0.0001$, Fig. 2). This prediction scheme could be valuable to reduce the need for complete ALN dissection and to properly select patients with high risk of distant metastasis for subsequent targeted therapy.

The main limitation of this study was subtyping of luminal breast cancer into A and B subtypes based on expression status of PR but not on expression status of the proliferation marker; Ki-67. However; recent studies have reported that absence of PR expression is associated with poor prognosis of breast cancer and proposed that PR-negative breast cancer should be classified as luminal B subtype [29,30]. Providing initial information about the intrinsic subtypes of breast cancer in Upper Egypt was one of the strong points of this study.

Conclusion

Breast cancer is no longer a single but rather a heterogeneous disease with diverse clinical, biological, pathological and

molecular features. Risk assessment of metastatic potential of this disease is important for therapeutic and prognostic implications. HER2 but not ER or PR expression was strongly associated with increased risk of breast cancer metastasis irrespective to tumor stage and grade and it had a strong validity to predict ALN metastasis in both univariate and multivariate regression analyses. The model using tumor size and HER2 expression status was able to accurately predict ALN metastasis with AUC of 86% at ROC curve analysis.

Conflict of interest

The author declared that there is no conflict of interest.

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