RESEARCH ARTICLE

Possible Prognostic Role of HER2/Neu in Ductal Carcinoma In Situ and Atypical Ductal Proliferative Lesions of the Breast

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Abstract

HER2/neu is a well-established prognostic and predictive factor for invasive breast cancer. However, the role of HER2/neu in ductal breast carcinoma in situ (DCIS) is debated and recent data have suggested that it is mainly linked to *in situ* local recurrence. Although molecular data suggest that atypical ductal hyperplasia (ADH) and duct carcinoma in situ (DCIS) are related lesions, albeit with vastly different clinical implications, the role of HER2/neu expression in atypical ductal hyperplasia is not well defined either. The aim of this study was to evaluate over expression of HER2/neu in DCIS and cases of ADH in comparison with invasive breast carcinoma. Archival primary breast carcinoma paraffin blocks (n=15), DCIS only (n=10) and ductal epithelial hyperplasia and other breast benign lesions (n=25) were analyzed for HER2/neu immunoexpression. Follow up was available for 40% of the patients. HER2/neu was positive in 80% of both DCIS and invasive carcinoma, and 67% of atypical ductal hyperplasia (ADH) cases. Thus at least a subset of patients with preinvasive breast lesions were positive, which strongly suggests a role for Her2/neu in identifying high-risk patients for malignant transformation. Although these are preliminary data, which need further studies of gene amplification within these patients as well as a larger patient cohort with longer periods of follow up, they support the implementation of routine Her2/neu testing in patients diagnosed as pure DCIS and in florid ADH.

Keywords: HER2/neu - duct carcinoma - DCIS - ADH - malignant transformation

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Introduction

The importance of HER2/neu in cancer has been of recent considerable interest, both in its role as a prognostic indicator and as a predictor of response to therapy. HER2/neu overexpression/amplification is seen more frequently in ductal carcinoma in situ (DCIS), particularly high-grade ductal carcinoma in situ (50-60%) (Borgquist et al., 2015), with an over all reported incidence of 13-56% of ductal carcinoma in-situ while in invasive ductal carcinoma of the breast it is reported in around 25-30% (Latta et al., 2002). Patients with HER2 /neu positive metastatic lesions appear to have more aggressive clinical course (Bezwoda 2000). Molecular data suggested that atypical ductal hyperplasia (ADH) and duct carcinoma in situ (DCIS) are related lesions, however, these 2 diagnoses have vastly different clinical implications. Patients with incompletely excised DCIS are at 10-fold-increased risk of developing invasive ductal carcinoma (IDC) at the involved site. In contrast, ADH confers a lower (4- to 5-fold) generalized increased risk of invasive breast cancer and is managed by close observation with or without chemopreventive hormone therapy. (Johnson and Collins, 2009; Christopher et al., 2013). The significance of HER2 overexpression in DCIS and in ADH has yet to be elucidated as some authors suggest a low risk for progression and invasion (Borgquist et al., 2015) while others suggests a more significant role but not as an independent prognostic factor (Al Mansour et al., 2013).

Aim of the Work: The current study aimed to evaluate the over expression of HER 2/neu in duct carcinoma in situ versus benign proliferative ductal epithelial changes of the breast lesions to shed light on the high risk patients with preinvasive lesions.

Materials and Methods

A retrospective study of 50 archival formalin fixed paraffin embedded sections of breast lesions of adult Egyptian female patients collected from Beni suef University Hospital during the period of January 2014-November 2015. Ten cases were DCIS (removed by localized excision with safety margins), 15 cases were from cases of IDC (removed by modified radical

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mastectomy) and 25 cases were ADH with adenosis and other benign lesions (removed by localized excision).

Available clinical data: For each case, the following clinical data were collected from the patients' records: age of the patient, breast side, site, type of surgical interference and pathological type of the lesions. Follow up was available for 20 patients, 15 from the benign proliferative lesions group and 5 from the DCIS group. No follow up data was available for the invasive duct carcinoma group in our hospital as all patients were referred to an oncology center.

Materials & Methods

Histopathological and immunohistochemical evaluation: In this work, the collected lesions were prepared from paraffin blocks. Serial sections from each tissue block were cut at 4 microns thickness. Multiple serial sections were stained by H&E for histopathological evaluation and selection according to the inclusion criteria (Pinder and Ellis, 2003). Two pathologists did the review and scoring of the cases independently.

Diagnostic criteria for ADH/ DCIS: Target lesions included any hyperplastic lesion (three or more cells above a basement membrane complex). ADH was defined as a uniform population of small or medium-sized, round, cuboidal or polygonal hyperchromatic cells, which are regularly arranged. Geometric spaces are present and, in the cribriform type, the cells are arranged at right angles to the bridges formed. DCIS was defined as a proliferation of malignant epithelial cells within the breast parenchymal structures with no evidence of invasion across the basement membrane.

HER2/neu immunostaining evaluation: Sections were prepared for immunostaining using monoclonal anti-human HER2/neu antibody and assessed using scoring system (Hercep Test Score) (Jimenez et al., 2001).

Positive control: Parallel positive sections of positive (+3) HER-2/neu breast duct carcinoma, cases were served as a positive control for each set of slides.

Negative control: Sections untreated with primary antibody (HER-2/neu) were considered as negative controls for each set of slides.

Target Score (Hercep Test Score): Score (0): Completely negative staining. Score (+1): Faint equivocal incomplete staining of the membrane (< 10% of malignant cells). Score (+2): Weak to moderate incomplete membranous staining in > 10 % of the tumor cells or complete membranous staining in <10 % of the tumor cells. Score (+3):- Strong staining of the complete membrane creating a fishnet pattern (in > 10% of malignant cells). Both scores 0 and +1 categorized as negative, tumors with scores of +2 or +3 were considered positive (HER-2/neu Protein overexpression). A cytoplasmic staining alone was considered non-specific and negative.

Other Immunohistochemical Markers

CK5/6 was used to differentiate between cases with usual ductal hyperplasia and ADH.

Alpha smooth muscle actin (α SMA) to assess the presence of myoepithelial cells to prove or exclude invasion.

ER and PR was performed for cases in the malignant group (DCIS and IDC)

Statistical Analysis

Analysis of significant clinico-pathological variables, as well as, tissue biomarkers was performed using the Qui-Square test and level of significance (P-value) was 0. 05.

Table 1. HER2/neu Overexpression in the Studied Cases

Pathological diagnosis	Number	Negative HER2	Positive HER2	p value
FCD , UDH	15 (30%)	11 (74%)	4 (26%)	> 0.05
Sclerosing adenosis and ADH	6 (12%)	2 (33%)	4 (67%)	> 0.05
Fibroadenoma	4 (8%)	4 (100%)	0 (0%)	< 0.05
DCIS	10 ((20%)	2 (20%)	8 (80%)	< 0.05
IDC	15 (30%)	3 (20%)	12 (80%)	< 0.05

Results

This study included fifty breast lesions from adult Egyptian females aged between 28-62y with a mean age of 42y. Breast lesions were previously surgically treated either by localized excision with a safety margin in 10 cases (20%), modified radical mastectomy (MRM) in 15 cases (30%) or localized excision in 25 cases (50%). Histologically cases were diagnosed as benign lesions

Table 2. Scoring of HER2/neu among the Benign and Malignant Lesions

Histopathological type		HER2 / neu score				Total
		0	1+	2+	3+	
Fibroadenoma	No. of cases	4	0	0	0	4
	% of Total	8%	0	0	0	8%
FCD with UDH	No. of cases	11	0	4	0	15
	% of Total	22%	0	8%	0	30%
ADH with adenosis	No. of cases	2	0	2	2	6
	% of Total	4%	0	4%	4%	12%
DCIS	No. of cases	2	0	2	6	10
	% of Total	4%	0	4%	12%	20%
IDC	No. of cases	3	0	4	8	15
	% of Total	6%	0	8%	16%	30%
Total	No. of cases	22	0	12	16	50
	% of Total	44%	0	24%	32%	100%

in 25 cases [15 cases with fibrocystic disease (FCD) with usual ductal hyperplasia (UDH), 6 cases ADH with adenosis, 4 cases with fibroadenoma] and 25 malignant cases (10 cases DCIS "5 cases were predominantly cribriform type, 3 were predominantly solid type and 2 were predominantly comedo type" while 15 cases were diagnosed as IDC).

Within the studied groups; Her2/neu over expression (score ≥ 2+) was noted in 80% of the IDC cases, 80% of the DCIS cases, in 16% of the cases with ADH and 16% of the cases with fibrocytic disease with usual ductal hyperplasia. None of the fibroadenoma cases (0/4) showed any expression of HER2 (Table 1). Twenty four out of the 28 HER2/neu positive cases (85%) were located in the left side of the breast.

Within the malignant group (DCIS & IDC) all the IDC cases with positive HER2/neu score showed negative estrogen and progesterone receptors while the 2 negative HER2/neu cases showed moderate and strong positivity respectively. Only one DCIS case within our cohort showed moderate positivity for both ER and PR, such case was HER2/neu negative. The HER2/neu score correlated significantly within the malignant groups (DCIS &IDC) in contrast to the ADH and UDH cases where no significant correlation with the score was found. (Table 2) & (Figure 1). In the DCIS group there was no significant correlation between the histological type and the HER2 expression score.

Follow up data was available for 5 patients within the DCIS group, 4 of them showed HER2/neu positive overexpression and one was negative. One of the positive cases with DCIS (score 3+) showed lymph node metastasis after one year. In the benign group, follow up of 15 patients was available as follows: 2 cases with fibroadenoma, 9 in the FCD-UDH group and 4 in the sclerosing adenosis and ADH group. One of the patients within the ADH/sclerosing adenosis group with HER2/neu positive overexpression (score3+) presented after 15 months with breast mass diagnosed as DCIS with an early invasive component.

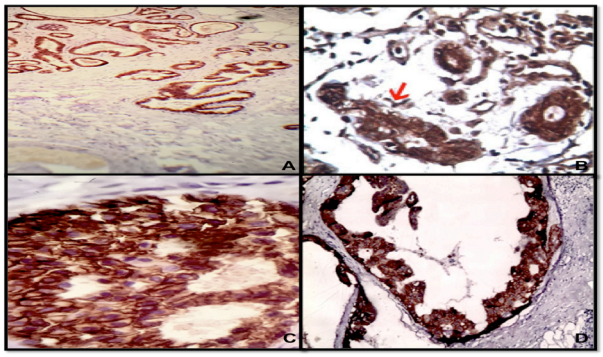

Figure 1. HER 2/neu Overexpression among Benign and DCIS of Studied Cases. (A): low power magnification (x100) showing HER2 expression (score2+) in a case of FCD-UDH. (B): High power magnification (x400) showing HER2 expression (score 3+) in sclerosing adenosis (red arrow). (C): High power magnification (x400) showing HER2 expression (score 3+) in a case of DCIS. (D) High power magnification (x400) showing HER2 expression (score 3+) in ADH

Discussion

Female breast carcinoma affects all socioeconomics levels in developing as well as developed countries (Abdurrahman et al., 2013). Assay for HER2/neu overexpression and/or amplification is a routine part of the evaluation of breast carcinomas because it is predictive of who might benefit from HER 2/neu-directed therapies (Mitri et al., 2012; Abdelhamid et al., 2014; Doval et al., 2015), however its role as a predictor of aggressive behavior in non invasive and atypical breast lesions is not well established (Sapino et al., 2013; Drakaki and Hurvitz, 2015). In this study, the prevalence of HER 2/neu immunoreaction in malignant lesions was higher than what was reported in other studies (80% in IDC and DCIS in our group versus an average of 20% in IDC and 60 % in DCIS in most large studies (Krishnaswamy et al., 2013; Al Mansour et al., 2013; Owens et al., 2004). As for HER 2/neu expression in benign breast lesions, we noticed over expression in 8 out of 25 (31.25%) benign patients, 26 % of them had the histological picture of fibrocystic disease. These findings are greater the study of Stark et al (2000) where 9.5% of their benign lesions exhibited HER2/neu score ≥ 2+ while 4.5% showed low level amplification. Others noticed over expression of HER2/neu in 10.9% of apocrine metaplasia and 55.6% of apocrine change within sclerosing adenosis lesions (Abdel-Ghani and Ghada 2002), while in our study we found overexpression in 4 patients (66.66 %) out of 6 cases of ADH with sclerosing adenosis. We believe the discrepancy in the prevalence, more in our group of patients, was mainly due to the small number of cases as well as the lack of FISH/CISH studies for cases with score 2+. However, cases with score 3+ were evident in both DCIS group and in the group with atypical ductal hyperplasia in our study group. Another important factor to be considered is the difference in the antibodies used (monoclonal versus polyclonal) as well as the technique applied (frozen versus paraffin fixed) (Cohen et al., 1989; Fendly et al., 1990; Tsutsumi et al., 1990; Rosen 1997; Sapino et al., 2013). Even though, with these variations we noticed that with the transition from purely benign lesions to malignant ones; the incidence of scoring grade was increased, in such cases inappropriate signalling may occur as a result of receptor overexpression or dysregulation of receptor activation that may lead to increased/uncontrolled cell proliferation, decreasing apoptosis and leading to malignant transformation and enhancement of invasion (Bezwoda, 2000). The overexpression of HER2/neu in both invasive and associated DCIS may point to the maintenance of underlying genetic alteration during transition from intraductal to invasive carcinoma and that intraduct carcinoma precedes the invasive phase, supporting the hypothesis that HER2/neu activation most likely occurs at an early stage of human breast carcinoma (Krishnaswamy et al., 2013) that could be of significant prediction for microinvasion (Sibel et al., 2014).

Conclusion: HER 2/neu is over expressed among invasive breast tumours and some cases with benign lesions as well as DCIS, the expression of HER2 / neu in the preinvasion (DCIS, ADH, adenosis) lesions could

have role in uncontrolled proliferation that may point to the maintenance of underlying genetic alteration during transition from ADH, adenosis, DCIS to invasive carcinoma supporting the hypothesis that HER2/neu activation most likely occurs at an early stage of human breast carcinoma. Patients with overexpressed receptors in non-invasive lesions could be those of highest risk of malignant transformation, even invasion and those cases might need further management rather than just surgical excision.

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