The Association Of Her-2/Neu Overexpression In Relation With P53 Nuclear Accumulation, Hormonal Recceptor Status And Common Clinicopathological Prognostic Parameters In A Series Of Egyptian Women With Inasive Ductal Carcinoma

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Abstract

Aim of the present study: to determine the association of HER-2/neu and p53 as well as hormonal receptor status with conventional common pathologic parameters in invasive ductal carcinoma of breast.

Methods: expression of HER-2/neu and p53 was examined immunohistochemically and significance was studied in relation with HR status and other clinicopathological parameters.

Results: 26%, were positive for HER2, there was a significant association between HER-2 and the accumulation of nuclear p53 (p=0.0017) and the increased size of tumor (p=0.0115) as well as the high grade tumors (p=0.0001) also there was an inverse significant association with ER (p=0.0001) and PR status (p=0.0003) but no significant association as regard number of the invaded LN, tumor necrosis and menopause.

Conclusion: Her-2/neu may be a powerful predictor of poor prognosis as was directly correlated with p53, tumor size, and grade and inversely correlated with HR status, of breast carcinoma.

INTRODUCTION

Breast cancer is a heterogeneous disease with variable biological and clinical characteristics. The racial influence in invasive breast cancer in terms of age at presentation, clinico-pathological features, and outcome of treatment has been widely reported. It has been established that breast cancer in many Asian and African countries tends to affect younger females, presents in advanced stage with poor prognostic features, and has worse outcome when compared to their counterparts in the Western countries. There is no doubt that the lack of early detection program and awareness contribute to advanced presentation, however, it is unclear why these carcinomas involving young females show higher biological aggressiveness in terms of poor differentiation and lack of steroid receptor expression [1]. Descriptions of prognostic factors in breast cancer have exploded over the past several years. Pathologists have played a major role in identifying different histological and immunohistochemical markers that have a direct bearing on both the treatment and behavior of breast cancer [2].

Besides several prognostic factors like tumor size, histological grade, steroid hormone receptor status, DNA ploidy, or lymph node status, the statues of HER-2/neu (human epidermal growth factor receptor 2) probably serves as an additional parameter. HER-2/neu proto-oncogene is involved in the regulation of normal cell growth and division and is expressed at low levels in many normal epithelial cells [3]. HER-2/neu proto-oncogene encodes an 185KD glycoprotein with tyrosine kinase activity. Over expression of this gene either due to gene amplification and/or increased transcription has been observed in a variety of cancers and has been associated with more aggressive disease and a poor

clinical prognosis in 20-30% of patients with breast cancer [4]. Over-expression of HER2/neu in breast cancer and certain other tumors appears to correlates with poor survival and to be a central mechanism that may be partly responsible for cellular progression of the neoplastic phenotype; moreover, HER-2/neu may potentiate tumorigenesis by inducing tumor cell resistance to host defense mechanisms [5] as it induces resistance to tumor necrosis factor (TNF), which causes cancer cells to escape from host immune defenses [6] and appears to result in reduced sensitivity to immune effectors killing [7].

The aim of this study is to determine the association of HER-2/neu over expression in relation with p53 nuclear accumulation, hormonal receptor status as well as conventional common pathologic parameters in a series of Egyptian women with invasive ductal carcinoma of breast.

PATIENTS AND METHODS

Fifty tumor samples from patients diagnosed as infiltrating ductal carcinoma were included in the present study, collected from the pathology department, Damanhour Medical Institute, the General Organization for teaching Hospitals and Institutes. All patients had operable tumors with no evidence of distance dissemination at time of diagnosis and underwent modified radical mastectomy as well as removal of axillary lymph nodes.

All tissues were fixed in 4% buffered neutral formaldehyde at room temperature for no more than 24 hrs. All tumor tissue and lymph nodes were examined microscopically. Histology was assessed for tumor subtypes, invasiveness, nuclear and histological grades and lymph node status. Carcinomas were classified on the basis of the H&E stained slides; all cases were invasive ductal carcinoma not otherwise specified (NOS) type and they were graded according to the Scarff-Bloom-Richardson system of the World Health Organization (WHO) classification [8].

IMMUNOHISTOCHEMICAL ANALYSIS

One 4-μm section from each submitted paraffin block was first stained with haematoxylin and eosin in order to verify the presence of an adequate number of invasive ductal carcinoma cells and verify the quality of fixation as sufficient for immunohistochemical analysis. Serial sections (4-μm) were prepared from the selected blocks and float mounted on adhesive coated glass slides for immunostaining. Positive control sections were included in

each run. Sections were processed using a citrate buffer for antigen retrieval [2].

The status of HER2 (DA 485 dil 1:1500) and p53 (DO7 dil 1:200), estrogen (6F11 dil 1:40) and progesterone receptors (1□6 dil 1:30) was determined by immunohistochemistry. Immunostaining was performed with a Nexus automated immunostainer (Ventana, Illkirch, France). Sections were scored semiquantitatively by light microscopy by three pathologists. For estrogen and progesterone receptors and p53, a threshold of 10% of stained nuclei was considered positive. For HER-2/neu, overexpression was defined as more than 10% of cells showing complete membrane staining with high intensity.

RESULTS

The 50 cases included in the present study ranged from 26 to 59 ys of age. Thirty-five cases (70%) were pre-menopausal and fifteen (30%) were post-menopausal. Five cases (10%) had a tumor size <2cm, twenty-seven cases (54%) had a tumor size 2-5 cm, while eighteen cases (36%) had a tumor size >5cm. In the present study all cases were presented with lymph node metastasis; seventeen cases (34%) had <10 LN invasion and thirty-three cases (66%) had >10LN invasion. As regard tumor grade; 5 cases (10%) were grade I, 27 cases (54%) were grade II and 18 cases (36%) were grade III. Tumor necrosis was marked in 2 cases (4%), moderate in 10 cases (20%) and absent in 38 cases (76%).

HER-2 over expression: HER-2/neu positive immunostaining was observed in 13 cases (26%) the staining reaction when present was cytoplasmic and along the cell membrane; nuclei were clearly negative (Figure 1: a, b & c).

Figure 1

Figure 1: a- HER-2/neu positive immunostaining (X100), b- HER-2/neu staining reaction when present was cytoplasmic and along the cell membrane (X400), c- nuclei were clearly negative (X200) & d- P53 accumulation presented as positive nuclear staining reaction (X200).
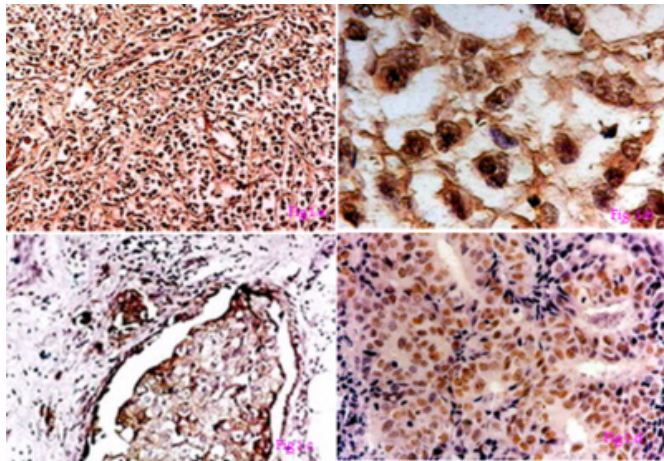
Her-2/neu over expression in relation to Hormonal status: ER nuclear immunoreactivity was positive in 31 cases (62%) , where 30 cases (60%) of these were HER-2 negative on the other side 19 cases (38%) were ER negative, 12 cases (24%) out of them were positive for HER-2 cellular accumulation. Statistically there is a highly significant association between HER-2 cellular accumulations and loss of ER nuclear positive immunostaining (p=0.0001). As regard the PR immune-staining 21 cases (42%) were PR positive, all of which were negative for HER-2 immuno-staining. Statistically there was also significant association between HER-2 cellular accumulation and PR negative immuno-stainning (p=0.0003).

HER-2/neu over expression in relation to P53: nuclear accumulation of p53 (Figure 1: d) was recorded in 17 cases (34%); there was a significant association between HER-2 over expression and the accumulation of nuclear p53 (p=0.0017).

HER-2/neu in over expression relation to Tumor size: Most of the HER-2 positive immunostaning were belonging to tumor sized >5cm in diameter; statistically there is a significant association between HER-2 positive immunostaining and the increased size of tumor (p=0.0115).

HER-2/neu over expression in relation to Tumor Grade: There was a highly significant association between HER-2 accumulation and the high grade tumors (p=0.00001).

HER-2/neu over expression in relation to Number of invaded Lymph Nodes: There was no statistical significant associated between HER-2 positive immunostaining and the number of the invaded lymph nodes (p=0.3337).

HER-2/neu over expression in relation to Tumor Necrosis: There was no significant association between the presence of HER-2 immunostaining and the degree of tumor necrosis (p=0.5881).

HER-2/neu over expression in relation to Menopausal Status: Finally there was also no significant association between HER-2 accumulation and the menopausal status (p=0.020).

Figure 2

Table 1: HER-2 cellular accumulation in relation to P53, hormonal status and the conventional prognostic variables of breast carcinoma

Variable	HER-2 +ve N	HER-2 +ve %	HER-2 -ve N	HER-2 -ve %	Total N	Total %	Chi-square	p-value
1. P53							9.7170	0.0017
◆P53 +ve	9		8		17	34%		
◆P53 -ve	4		29		33	66%		
2. Estrogen Recep.							21.9917	0.00001
◆ER +ve	1		30		31	62%		
◆ER –ve	12		7		19	38%		
3. Progesterone Recap.							12.7213	0.0003
◆PR +ve	0		21		21	42%		
◆Pr -ve	13		16		29	58%		
4. Tumor size:							8.9012	0.0115
◆<2 cm	0	0%	5	10%	5	10%		
◆2-5 cm	4	8%	23	46%	27	54%		
◆>5 cm	19	38%	9	18%	18	36%		
5. Tumor Grade:							18.1412	0.00001
◆Grade I	0	0%	5	10%	5	10%		
◆Grade II	2	4%	25	50%	27	54%		
◆Grade III	11	22%	7	14%	18	36%		
6. Number of LN:							0.9341	0.3337
◆<10LN invaded	3	6%	14	28%	17	34%		
◆>10LN invaded	10	20%	23	46%	33	66%		
7. Tumor necrosis:							1.0615	0.5881
◆Non	11		27		38	76%		
◆Moderate	2		8		10	20%		
◆Marked	0		2		2	4%		
8. Menopause:							4.7570	0.020
◆Pre-menopause	6	12%	29	58%	35	70%		
◆Post-menopause	7	14%	8	16%	15	30%		

P≤0.01 is significant

DISCUSSION

The aggressive biological behavior of invasive and metastatic cancer is considered to be the most insidious and life threatening aspect for breast cancer patients. It is mostly the result of changes in many molecular characteristics of tumor cells, including alterations in mechanisms controlling

cell growth and proliferation [9]. The biology of breast cancer remains poorly understood, although knowledge about individual prognostic factors provides limited information [10]. A wide variety of morphology-based and molecular-based prognostic factors and tumor markers have been studied as to their potential to predict disease outcome in breast cancer. An expanded understanding of the biology of breast cancer has led to the identification of the HER-2/neu receptor as an important growth factor [12]. This receptor possesses intrinsic tyrosine kinase activity and has been associated with aggressive biological behavior and poor clinical outcome Following the original study by Salmon and coworkers in 1987 [13] and many investigators have considered the prognostic potential of the HER-2/neu gene and protein in breast cancer [14]. The HER-2/neu proto-oncogene encodes a growth factor receptor that is over expressed in 20-30% of metastatic breast cancers. This over expression is associated with decreased survival and decreased relapse-free periods. HER-2/neu is an important gene in breast cancer because who whose cancer shows HER-2/neu amplification generally have a poor prognosis. HER-2/neu gene amplification is also a predictive marker of responsiveness to selected forms of therapy. Clinical studies have demonstrated that alterations in HER-2/neu predict poor prognosis for breast cancer and are associated with features of tumor aggressiveness, such as absence of estrogen and progesterone receptors, high rate of cellular proliferation, advanced tumor stage, large tumor size, and young age at diagnosis [15].

Wild-type p53 plays two major roles in cell function: first, it regulates the checkpoints G1 to S and G2 to M of the cell cycle, through regulation of transcription of p21 and other genes; second, it induces apoptosis after genotoxic damage. Simultaneous over expression of c-erbB-2 and p53 has been reported to be prognostically unfavorable in breast cancer [16]; the development of p53 abnormalities might accompany or precede the development of Her-2/neu over expression [17]. Tumors with both HER-2 over-expression and p53 protein accumulation were reported in several studies [18 ,19] and patients with such tumors were found to have poor prognosis. In the present study findings reported indicated that both HER2 over-expression and p53 protein are significantly associated. On the other hand, some studies have shown a better prognosis in patients with breast cancers with HER-2/neu over expression and p53 protein accumulation as they suggested that differences may reflect

the effect of various therapeutic regimens [2].

HER-2/neu amplification and ER/PR alterations are early events in breast carcinogenesis [14]. An inverse association had been found between Her-2/neu amplification/ over expression and the presence of receptors for steroid hormones estrogen and progesterone in both clinical correlative studies and experimental models; thus the higher the level of Her-2/neu over expression the lower the corresponding ER level [20]. Our data also demonstrated an inverse correlation between Her-2/neu over expression and ER status, also there was an inverse correlation between Her-2/neu and PR status that most likely occur because suppression of ER expression leads to reduced expression of PR.

HER-2 status has been used as a marker for breast cancer prognosis in addition to the conventional classic factors as lymph node status, tumor size, type and grade [21]. In the present study Her-2/neu over expression was significantly associated with the tumor size (p=0.0115) and tumor grade (p=0.00001). Others studies data indicating the association of HER-2/neu positively with large tumors [22,23].

Unexpected finding; there was no significant association between the Her-2/neu over expression and the number of invaded lymph nodes; in the present study all cases had lymph nodes involvements. Others studies showed a direct association between HER-2 and metastatic involvement of Lymph nodes [24,25] meanwhile; Sutterlin et al ,2000 reported that there was no significant relation between HER-2 over expression and lymph node involvement [26]. Korkolis et al, 2004 added in their study that deregulation of HER-2 oncogene might characterize a subgroup of node negative patients with poor prognosis who could benefit from an aggressive adjuvant therapy [27].

Cell death relates to the inactivation of HER-2/neu receptor in breast cancer [28]. Others study mentioned a significant relation between HER-2/neu down regulation and tumor necrosis [29, 30]. Another unexpected finding in the present study; the absence of significant association between the Her-2/neu over expression and the grade of tumor necrosis. Zhou et al, 2000 suggested that HER-2/neu constitutively activates the anti apoptotic cascade to confer resistance to TNF (Tumor necrosis factor) on cancer cells and reduce host defenses against neoplasia [6]. Egeblad & Jattela, 2000 study's data didn't support the role of ErbB receptors in

regulation if cell death [31].

Finally, there was no significant association between Her-2/neu over expression and menopausal status. Same finding was reported in Adebamowo and coworkers study [32]. In Ray et al study; HER-2/neu over expression was significantly higher among post menopausal in comparison with pre-menopausal [24].

CONCLUSION

The study had indicated that Her-2/neu may be a powerful predictor of poor prognosis as it's over expression was strongly correlated with p53 nuclear accumulation, absence of hormonal receptors immunoreactivity and unfavorable clinico-pathological variables of breast carcinoma in Egyptian females. Meanwhile no significant associated for both as regard number of invaded lymph nodes and the menopausal status.

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References

1. Al-Moundhri M, Nirmala V, Al-Mawaly K et al. Significance of p53, Bcl-2 and Her-2/neu protein expression in omani arab females with breast cancer. Pathol Oncol Res vol.9 Nov. 2003, 226-231.
2. Yamashita H, Nishio M, Toyama T et al. Coexistence of HER-2 over-expression and p53 protein accumulation is a strong prognostic molecular marker in breast cancer. Breast Cancer Res. 2004, 6:R24-R30. http://breast-cancer-research.com/content/6/1/R24
3. Meenakshi A, Kumar RS, Ganesh V et al. Immunohistochemical assay (IHC) to study C-erbB-2 status of breast cancer using monoclonal antibody CIBCgp185. Hum Antibodies. 2003; 12(4):123-7.
4. Diaz NM. Laboratory testing for Her-2/neu in breast carcinoma: An evolving strategy to predict response to targeted therapy. Cancer Control; Sep-Oct 2001, vol.8 no5; 415-418.
5. Hudziak RM, Lewis GD, Shalaby MR et al. Amplified expression of the HER-2/ErbB2 oncogene induces resistance to tumor necrosis factor alpha in NIH3T3 cells. Proc Natl Acad Sci USA 1988 July; 85(14):5102-5106.
6. Zhou BP, Hu MC, Miller SA et al. HER-2/neu blocks tumor necrosis factor induced apoptosis via the Akt/NF-kappa B pathway. J Biol Chem. 2000, Mar 17; 275(11):8027-31.
7. Rosenblum MG, Horn SA & Cheung LH. A novel recombinant fusion toxin targeting HER-2/neu over expressing cells and containing human tumor necrosis factor. Int J Cancer, 2000 Oct 15;88(2):267-73.
8. Scarff RW and Torloni H. Histological typing of breast tumors. 1968 International histological classification of tumors, no2 vol2; World Health Organization, Geneva, pp 13-20.
9. Hlupic L, Jakic-Razumovic J, Bozikov J et al. Prognostic value of different factors in breast carcinoma. Tumori 2004 Jan-Feb; 90(1): 112-9.
10. Sorli T, Perou CM, Tibshirani R et al. Gene expression patterns of breast carcinomas distinguishes tumor subclasses with clinical implication. PNAS Sep 2001 Vol 98 no.19:10869-10874.
11. Winters ZE, Leek RD, Bradurn MJ et al. Cytoplasmic p21 WAF1/CIP1 expression is correlated with HER-2/neu in breast cancer and is an independent predictor of prognosis. Breast Cancer Res. 2003, 5:R242-R249. http://ccforum.com/content/5/6/R242
12. Salmon DJ, Clark GM, Wong SG et al. Human breast cancer. Correlation of relapse and survival with amplification of the Her-2/neu oncogene. Science 1987; 235:177-182.
13. Ross JS and Fletcher JA. The Her-2/neu oncogene in breast cancer: Prognostic factor, prediction factor and target for therapy. The Oncologist, Vol 3 , no.4, 237-252 ; August 1998.
14. Huang WY, Newman B, Millikan RC et al. Risk of breast cancer according to the status of Her-2/neu oncogene amplification. Cancer epidemiology biomarkers & Prevention vol.9, 65-71, Jan 2000
15. Nieto Y, Cagnoni PJ, Nawaz S et al. Evaluation of the predictive value of Her-2/neu over expression and p53 mutations in high risk primary brest cancer patients treated with high dose chemotherapy and autologous stem cell transplantation. J Clin Oncol vol.18 issue 10 (May) 2000:2070-2080
16. Guerra I, Algorta J, Díaz de Otazu R. Immunohistochemical prognostic index for breast cancer in young women. Molecular Pathology 2003;56:323-327 http://mp.bmjjournals.com/cgi/content/full/56/6/323
17. Smith CA, Pollice AA, Gu LP et al. Correlations among p53, Her-2/neu and ras over expression and aneuploidy by multiparameter flow cytometry in human breast cancer: Evidence for a common phenotypic evolutionary pattern in infilterating ductal carcinoma. Clinical Cancer Res. Vol.16 : 112-126, Jan 2000.
18. Marks JR, Humphrey PA, Wu K et al. Over expression of p53 and HER-2/neu proteins as prognostic markers in early stage breast cancer. Ann Surg 1994 Apr; 219(4):332-41.
19. Tsutsui S, Ohno S, Murakami S et al. Prognostic significance of the co-expression of p53 protein and c-erbB2 in breast cancer. Am J Surg. 2003 Feb;185(2):165-7.
20. Konecny G, Pauletti G, Pegram M et al. Quantitive associated between Her-2/neu and steroid hormone receptors-positive primary breast cancer. JNCI vol.95 No.2, 142-153, Jan 15, 2003.
21. Thor A. Are patterns of Her-2/neu amplification and expression among primary and regional metastasis indicative of distance metastasis and predictive of herceptin response? JNCI vol 93 no 15:112-1121, August 2001.
22. Menard S, Frotis S, Castiglioni F et al. HER-2 as a prognostic factor in breast cancer. Oncology 2001; 61 suppl 2:67-72.
23. Swede H, Moysich KB, Winston JS et al. Variation of the prognostic significance of Her-2 expression in breast cancer according to tumor size. Breast J. 2003 Mar-Apr; 9(2):98-105.

24. Ray A, Sharma BK, Kaur S et al. Over expression of c-erbB2 oncoprotein and associated pathobiological factors in invasive primary breast cancer. Indian J Exp Biol. 2004Mar; 42(3):253-8.

25. Climent MA, Segui MA, Molina R et al. Prognostic value of HER-2/neu and p53 expression in node positive breast cancer. HER-2/neu effect on adjuvant tamoxifen treatment. Breast, 2001 Feb; 10(1):67-77.

26. Sutterlin MW, Haller A, Gassel AM et al. The correlation of c-erbB2 oncoprotein and established prognostic factors in human breast cancer. Anticancer Res. 2000 Nov-Dec; 20(6D):5083-8.

27. Korkolis DP, Tsoli E, Fouskakis D et al. Tumor histology and stage but not p53, Her-2/neu or cathepsin-D expressions are independent prognostic factors in breast cancer patients. Anticancer Res. 2004 May-June;24(3b):2061-8.

28. Nishikawa K, Satoh H, Hirai A et al. alpha-Tocopheryloxy butyric acid enhances necrotic cell death in breast cancer cell treated with chemotherapy agent. Cancer lett. 2003 Nov 10;201(1):51-6.

29. Bobrow LG, Happerfield LG, Gregory WM et al. Ductal carcinoma in situ: assessment of necrosis and nuclear morphology and their association with biological markers. J Path. 1995 Aug; 176(4):333-41.

30. Yagata H, Hariaga K, Suzuki M et al. Comedonecrosis is an unfavorable marker in node negative invasive breast carcinoma. Pathol Int 2003 Aug; 53(8):501-6.

31. Egeblad M and Jattela M. Cell death induced by TNF or serum starvation is indepent of erbB receptor signaling in MCF-7 breast carcinoma cells. Int J Cancer. 2000 June, 1; 86(5):617-25.

32. Adebamowo CA, Ogundiran A, Kang EE et al. Somatic p53 and HER-2/neu in Nigerian breast cancer. Proc Am Clin Oncol 22: page 885; 2003 (abstract 3557).

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