Original Article

Correlation of Her-2/Neu and Tp53 Expressions with Clinicopathologic Characteristics in Infiltrative Ductal Breast Carcinomas

Noushin Afshar Moghaddam[1], Parvin Mahsoni[1], Parvin Rajabi[1], Amir Pooyan Tabibi[1]

1. Dept. of Pathology, School of Medicine, Isfahan University of Medical Sciences, Isfahan, Iran.

ABSTRACT

Objective: **To review Her-2/neu and Tp53 status and their correlation with all other prognostic clinicopathologic features of infiltrative ductal breast carcinomas.**

Materials and Methods: **This cross sectional study was performed on 139 patients with infiltrative ductal breast carcinoma who were diagnosed between May 2000 and March 2006 at the surgery and pathology departments of Alzahra Hospital, Isfahan, Iran. Immunostaining (IHC) for Tp53 and Her-2/neu were performed on formalin-fixed, paraffin-embedded tissues based on an avidin-biotin-peroxidase complex technique. The relationship of these markers with clinicpathologic parameters including age, axillary lymph nodes status, tumor size and histological grade were evaluated.**

Results: **It was found out that Her-2/neu-positive cases were greater among metastatic lymph nodes than in patients without metastasis, however it was not significant (p=0.1). A significant association was also observed between Her-2/neu status and tumor grading (p=0.01). On the contrary, no association was found with other clinicpathologic parameters. In this study, Tp53 presentation in high-grade carcinomas was significantly more as compared to low grade ones (p=0.03). A significant association was also observed between Tp53 and tumor size (p =0.01). There was no association with menopausal status and lymph node status.**

Conclusion: **IHC determined that Her-2/neu and Tp53 expressions are not associated with nodal and menopause status. Conversely, a correlation was found between Her-2/neu, Tp53 expressions and high histological grade of tumor. However, to validate these findings, long-term prospective studies on patients' survival are necessary.**

Key words: Her-2 proto oncogene protein, Tp53 protein, Mammary ductal carcinoma, Lymphatic Metastasis, Menopause

Introduction

Clinical outcome of breast carcinoma is affected by prognostic and predictive factors. Prognostic factors are associated with either the metastatic or the growth potential of the primary tumor. Predictive factors are associated with the relative sensitivity and/or resistance to specific therapies (1-4). In the last few years, in addition to research on well-known clinicopathologic factors (such as patient age, tumor size, axillary nodes status and hormone receptors) there have been a lot of studies concerning the biological factors which affect tumor behavior and also explain the carcinogenesis in breast carcinoma. Her-2/neu (c-erb-B2) overexpression and Tp53 mutation are

Received: 20 January 2008
Accepted: 6 April 2008
Address communications to: Dr. Noushin Afshar Moghaddam, Department of Pathology, School of Medicine, Isfahan University of Medical Sciences, Isfahan, Iran.
Email: afsharmoghadam@mui.med.ac.ir

said to have an adverse effect on prognosis and also influence the response to therapy (5,6). The human epidermal growth factor receptor-2 (Her-2/neu) is located on chromosome 17q211 and encodes a 185 kda transmembrane glycoprotein that is homologous to the other three members of the family: Her-1, Her-3, and Her-4. The Her-2/neu gene amplification and/or protein overexpression occur in 14-30% of all breast cancer cases (7,8). The prognostic significance of Her-2/neu has remained a controversial issue yet (9). Several studies have suggested a direct correlation between Her-2/neu and poor clinical outcome in certain subsets of breast cancer patients (10,11) while some other studies did not report any clinical correlation (12,13).

The Tp53 gene is located on the short arm of chromosome 17. It encodes a 53-kd nuclear protein that can induce cell cycle arrest or apoptosis in response to DNA damage and its inactivation can lead to uncontrolled cellular proliferation. The wild-type p53 gene has a short half-life and is virtually undetectable in tissues. When mutated, the new conformationally altered p53 protein is stabilized and detectable on immunohistochemistry (14). Tp53 gene mutations have been reported in different human tumors. With regard to breast cancer, altered Tp53 has been identified in 15% of in-situ and 50% of invasive diseases. According to the higher proliferative and lower apoptosis rates due to loss of Tp53 function, altered p53 should therefore be associated with a worse clinical outcome (6). There is a high degree of variability which may be due to different immunohistochemical techniques, varying assessment of results for monoclonal antibody type (15). In the present study we tried to inspect the expression of Her-2/neu and Tp53 in relation to the clinicalpathologic features of the disease.

Materials and Methods

This cross-sectional study included 139 patients with breast infiltrative ductal carcinomas (IDCs) who were diagnosed between May 2000 and March 2006 at the surgery and pathology departments of Alzahra Hospital, Isfahan, Iran. Paraffin-embedded, formalin-fixed tissues were obtained from the pathology department. Samples were reviewed by a pathologist to ensure adequacy and to confirm that they were representative of the actual tumor. Immunostaining for Tp53 and Her-2/neu were performed on formalin-fixed, paraffin-embedded tissues based on an avidin-biotin-peroxidase complex technique. The additional sections with a thickness of 3 micrometer were prepared for immunohistochemical staining. After deparraffinization and hydration, slides incubated with 3% hydrogen peroxide for 20 minutes. Antigenic retrieval was done during incubation with molar citrate buffer 1 % (PH=6) in microwave oven for 20 minutes. Then, slides were incubated with antibody at room temperature. The monoclonal mouse anti-human p53 protein (clone Do-7) at a dilution of 1/50 and polyclonal rabbit anti-human Her-2 (code No.A0485) at a dilution of 1/250 (DAKO Corporation, Denmark) were used. Immunostaining was assessed with a Zeiss microscope at HPF (×400 overall magnification) and a field diameter of 0.46 mm. In IDC neoplastic cells populating the proportion of immunoreactive cells graded semi-quantitatively. Initial scoring was for 10 HPFs; however, in view of the homogeneous staining, this was reduced to 5 HPFs. The immunostaining of neoplastic cells were interpreted by two trained surgical pathologist who were blind about clinical outcomes of these patients. Scoring was based on the estimated proportion of tumor cells which stained positively: nuclear staining for Tp53 and cytoplasmic staining for Her-2. Cases were considered negative for a specific marker if 10% or less of the malignant cells stained for the antibody. If more than 10% of the malignant cells were stained with the antibody, cases were scored positive. The positive results for Her-2 were scored in three grades as follows: faint or blush staining involving a portion of the cytoplasmic membrane circumference in at least 10% of neoplastic cells (1+), weak but definitive staining of the membrane involving 100% of the circumference in at least 10% of neoplastic cells (2+) and strong positive staining of the membrane involving 100% of the circumference in at least 10% of neoplastic cells (3+) (16). In this study, the immunoreactivity was only graded for Her-2/neu. The relationship of these markers with clinicopathologic parameters including age, axillary lymph nodes status, tumor size and histological grade were evaluated. Data was analyzed using SPSS (version 10.0). The associations between tumor markers and patient characteristics were assessed with the Chi-square test.

Results

The mean age of studied patients was 50.7+ 17

years (ranging from 23 to 80 years old). According to menopausal status, they were divided into two age groups: 71 cases (51%) as pre-menopause and 68 cases (49%) as post-menopause. The histological grades were as follows: grade 1 in 29 cases (21%), grade 2 in 57 cases (41%) and grade 3 in 53 (38%) cases. According to tumor size, samples in our study were divided into two groups: 67 cases (48%) were equal or smaller than 50 mm and 72 cases (52%) were larger than 50 mm. The association between the expressions of Her-2 and Tp53 with clinicpathologic parameters is summarized in Tables 1-2. Compared to patients without metastases (39 cases), the total number of Her-2/neu-positive cases was greater among metastatic lymph nodes (55 cases). However, no statistically significant difference was observed (p=0.1). A significant association was also seen between Her-2/neu status and tumor grading (p=0.01). On the contrary, no association was found out for other clinicpathologic parameters such as tumor dimension and menopausal status. In this study, Tp53 presentation in high-grade or poorly-differentiated nuclear grade carcinomas was significantly more in comparison with low grade ones (p=0.03). A significant association was also observed between Tp53 and tumor size (p=0.01). On the other hand, no association was noted for menopausal status. In addition, TP53 was seen in node-positive cases (n=37) more than node-negative patients (n=25). However, there was no statistically significant difference (p=0.1).

Table 1. Correlation of Her-2(c-erb-B2) status with clinicpathologic factors

Characteristics	Her-2 score				p value
	Negative	1+	2+	3+	
Age					
Pre-menopause	24	15	7	25	0.2
Post- menopause	21	24	7	16	
Grade					
1	13	13	1	2	0.01*
2	17	14	9	17	
3	15	12	4	22	
Size					
<50 mm	24	22	7	19	0.8
>50 mm	21	17	7	22	
Lymph node status					
Negative	27	19	6	14	0.1
Positive	18	20	8	27	

Table 2: Correlation of Tp53 protein status with clinicpathologic factors

Characteristics	Tp53 score		p value
	Negative	Positive	
Age			
Pre-menopause	35		0.2
Post- menopause	41	27	
Grade			
1	10		0.03 *
2	21		
3	22	31	
Size			
<50 mm	18		0.01 *
> 50 mm	38	34	
Lymph node status			
Negative	25		0.1
Positive	36	37	

* Significant

Discussion

Immmunohistochemistry(IHC) is the most common method to evaluate Her-2 and Tp53 status in breast carcinoma. On the basis of some experts' studies, the proportion of Her-2-positive tumor cells in IHC methods has a wide range from laboratory to laboratory with an inter-observer variability (17,18). On the other hand, fixative type can affect the staining pattern of Tp53 in mammary carcinoma (19). Several attempts have been made to semi-quantitate immunohistochemical method through standardizing the technical procedure and reporting and using appropriate controls (20-22). Battifora et al have proposed a very advanced procedure which they referred to as Quicgel method (22). Although the idea is inventive, it may be too complex to be widely adopted. In this study, two parameters were evaluated in immunohistochemical preparations: the number of stained tumor cells and the reaction intensity. The first was expressed as percentage and the second was graded as 0, 1+, 2+, and 3+ (17,23,24). Although several complicated image analytic programs have been devised for this matter (25), but in most laboratories these estimations are done visually. The prognostic value of Her-2/neu has always been controversial. Studies showing a shorter overall survival for Her-2/neu-positive patients have been contradicted by others which failed to find such an association. In any case, Her-2/neu exact role as a predictive marker in breast cancer is still a matter of debate (26). Several possible reasons could account for this inconsistency. One frequently quoted reason could be the rather small sample size of many studies. However, probably the most important reason lies in the lack of standardized evaluation protocols in most of the earlier studies. In this retrospective study, we have assessed the Her-2/neu expression through IHC on 139 patients suffered from breast cancer. Compared with patients without metastases (39 cases), the total number of Her-R2/neu-positive cases was greater among metastatic lymph nodes (55 cases). This data is in substantial agreement with most of the reported data in literatures (26). However, no statistically significant difference was observed. A significant association was observed between Her-2/neu status and tumor grading. On the contrary, no association was noted with other clinicpathologic parameters such as tumor dimension and menopausal status. The findings of Traina et al and Al Ahwal et al are in agreement with ours (26,27), but in most of the studies including those conducted by Van de Vijver et al and Pestereli et al, a positive correlation between Her-2/neu expression and tumor size was found out. We thought that this may be due to population characteristics that we were dealing with or due to Her-2/neu antibody and scoring method (5,16,23,24,28).

Tp53 was another prognostic factor that was evaluated in our study. Mutations in theTp53 gene can occur in a number of ways including missense, nonsense, and frame shift mutations. They are missense mutations that usually result in an increased half-life of the protein product and then accumulation of the mutant Tp53 protein can be detected by immunohistochemistry (14). Most studies looking at the association between Tp53 mutation and survival have found a poorer prognosis with increased Tp53 expression (29,30). Others have found no difference or have even found an improved survival (31). One of the reasons for the conflicting results about the role of Tp53 mutations in breast cancer prognosis is the methodology used to detect them. Obviously the most sensitive and reproducible way to detect Tp53 mutations is DNA-sequence confirming, but to analyze as many cases as those existed in this study will be too expensive and time consuming. Recently some evidences have appeared in the literature indicating acceptable concordance between immunohistochemical techniques and mutational analysis (14). In this study, Tp53 presentation in high-grade or poorly differentiated nuclear grade carcinomas was significantly more as compared to low grade ones. Considering that loss of Tp53 function leads to higher proliferation and lower apoptosis rates altered Tp53 should therefore be associated with an aggressive clinical behavior (16). However, no statistically significant difference was observed.

Conclusion

To validate these findings, we recommend long-term prospective studies on patients' survival and there is also a great need to standardize these biomarkers assays and slide-scoring procedures.

References

1. Arykok AT, Onal BU, Han U. Expressions of cyclin D1, p53, bcl-2, and bax in infiltrative ductal carcinoma of the breast: correlations with clinicopathologic characteristics. Breast J 2006 Jul;12(4):391-2.

2. Porter-Jordan K, Lippman ME. Overview of the

biologic markers of breast cancer. Hematol Oncol Clin North Am 1994 Feb;8(1):73-100.

3. Hanna W, Kahn HJ, Trudeau M. Evaluation of Her-2/neu (erbB-2) status in breast cancer: from bench to bedside. Mod Pathol 1999 Aug;12(8):827-34.

4. Page DL, Jensen RA, Simpson JF. Routinely available indicators of prognosis in breast cancer. Breast Cancer Res Treat 1998;51(3):195-208.

5. Gurkan A, Erdogan G, Erdogan O, Pestereli E, Ogus M, Karaveli S, et al. Expression of c-erbB-2 and p53 in breast carcinoma patients: comparison with traditional prognostic factors and survival. J Int Med Res 2004 Sep;32(5):455-64.

6. Zellars RC, Hilsenbeck SG, Clark GM, Allred DC, Herman TS, Chamness GC, et al. Prognostic value of p53 for local failure in mastectomy-treated breast cancer patients. J Clin Oncol 2000 May;18(9):1906-13.

7. Hynes NE, Stern DF. The biology of erbB-2/neu/Her-2 and its role in cancer. Biochim Biophys Acta 1994 Dec 30;1198(2-3):165-84.

8. Riese DJ, Stern DF. Specificity within the EGF family/ErbB receptor family signaling network. Bioessays 1998 Jan;20(1):41-8.

9. Ariga R, Zarif A, Korasick J, Reddy V, Siziopikou K, Gattuso P. Correlation of her-2/neu gene amplification with other prognostic and predictive factors in female breast carcinoma. Breast J 2005 Jul;11(4):278-80.

10. Thor AD, Berry DA, Budman DR, Muss HB, Kute T, Henderson IC, et al. erbB-2, p53, and efficacy of adjuvant therapy in lymph node-positive breast cancer. J Natl Cancer Inst 1998 Sep 16;90(18):1346-60.

11. Ferrero-Pous M, Hacene K, Bouchet C, Le D, V, Tubiana-Hulin M, Spyratos F. Relationship between c-erbB-2 and other tumor characteristics in breast cancer prognosis. Clin Cancer Res 2000 Dec;6(12):4745-54.

12. Clark GM, McGuire WL. Follow-up study of Her-2/neu amplification in primary breast cancer. Cancer Res 1991 Feb 1;51(3):944-8.

13. Wright C, Angus B, Nicholson S, Sainsbury JR, Cairns J, Gullick WJ, et al. Expression of c-erbB-2 oncoprotein: a prognostic indicator in human breast cancer. Cancer Res 1989 Apr 15;49(8):2087-90.

14. Linjawi A, Kontogiannea M, Halwani F, Edwardes M, Meterissian S. Prognostic significance of p53, bcl-2, and Bax expression in early breast cancer. J Am Coll Surg 2004 Jan;198(1):83-90.

15. Sirvent JJ, Fortuno-Mar A, Olona M, Orti A. Prognostic value of p53 protein expression and clinicopathological factors in infiltrating ductal carcinoma of the breast. A study of 192 patients. Histol Histopathol 2001 Jan;16(1):99-106.

16. Rosai J. Ackerman's Surgical Pathology. 9 ed. Edinburg – London: Mosby; 2004.

17. Cserni G, Kalman E, Kulka J, Orosz Z, Udvarhelyi N, Krenacs T. [Quality control of Her-2 immunohistochemistry--results from a Hungarian study]. Magy Onkol 2007;51(1):23-9.

18. Fitzgibbons PL, Murphy DA, Dorfman DM, Roche PC, Tubbs RR. Interlaboratory comparison of immunohistochemical testing for Her-2: results of the 2004 and 2005 College of American Pathologists Her-2 Immunohistochemistry Tissue Microarray Survey. Arch Pathol Lab Med 2006 Oct;130(10):1440-5.

19. Fisher CJ, Gillett CE, Vojtesek B, Barnes DM, Millis RR. Problems with p53 immunohistochemical staining: the effect of fixation and variation in the methods of evaluation. Br J Cancer 1994 Jan;69(1):26-31.

20. Allred DC, Harvey JM, Berardo M, Clark GM. Prognostic and predictive factors in breast cancer by immunohistochemical analysis. Mod Pathol 1998 Feb;11(2):155-68.

21. Rhodes A, Jasani B, Balaton AJ, Miller KD. Immunohistochemical demonstration of oestrogen and progesterone receptors: correlation of standards achieved on in house tumours with that achieved on external quality assessment material in over 150 laboratories from 26 countries. J Clin Pathol 2000 Apr;53(4):292-301.

22.Riera J, Simpson JF, Tamayo R, Battifora H. Use of cultured cells as a control for quantitative immunocytochemical analysis of estrogen receptor in breast cancer. The Quicgel method. Am J Clin Pathol 1999 Mar;111(3):329-35.

23. Skaland I, Ovestad I, Janssen EA, Klos J, Kjellevold KH, Helliesen T, et al. Comparing subjective and digital image analysis Her-2/neu expression scores with conventional and modified FISH scores in breast cancer. J Clin Pathol 2008 Jan;61(1):68-71.

24. Dendukuri N, Khetani K, McIsaac M, Brophy J. Testing for Her-2-positive breast cancer: a systematic review and cost-effectiveness analysis. CMAJ 2007 May 8;176(10):1429-34.

25. Baddoura FK, Cohen C, Unger ER, DeRose PB, Chenggis M. Image analysis for quantitation of estrogen receptor in formalin-fixed paraffin-embedded sections of

breast carcinoma. Mod Pathol 1991 Jan;4(1):91-5.

26. Traina A, Agostara B, Marasa L, Calabro M, Zarcone M, Carruba G. Her-2/neu expression in relation to clinicopathologic features of breast cancer patients. Ann N Y Acad Sci 2006 Nov;1089:159-67.:159-67.

27. Al-Ahwal MS. Her-2 positivity and correlations with other histopathologic features in breast cancer patients--hospital based study. J Pak Med Assoc 2006 Feb;56(2):65-8.

28. van d, V, Peterse JL, Mooi WJ, Wisman P, Lomans J, Dalesio O, et al. Neu-protein overexpression in breast cancer. Association with comedo-type ductal carcinoma in situ and limited prognostic value in stage II breast cancer. N Engl J Med 1988 Nov 10;319(19):1239-45.

29.Levesque MA, Katsaros D, Yu H, Giai M, Genta F, Roagna R, et al. Immunofluorometrically determined p53 accumulation as a prognostic indicator in Italian breast cancer patients. Int J Cancer 1998 Apr 17;79(2):147-52.

30.Thor AD, Moore DH, II, Edgerton SM, Kawasaki ES, Reihsaus E, Lynch HT, et al. Accumulation of p53 tumor suppressor gene protein: an independent marker of prognosis in breast cancers. J Natl Cancer Inst 1992 Jun 3;84(11):845-55.

31. Gohring UJ, Scharl A, Heckel C, Ahr A, Crombach G. P53 protein in 204 patients with primary breast carcinoma--immunohistochemical detection and clinical value as a prognostic factor. Arch Gynecol Obstet 1995;256(3):139-46.