

Original Article

Correlation of ER, PR, HER- 2 and P53 Immunoreactions with Clinico-Pathological Features in Breast Cancer

Alireza Abdollahi, Sara Sheikhabaei, Somaye Safinejad, Issa Jahanzad

Dept. of Pathology, Imam Hospital Complex, Tehran University of Medical Sciences, Tehran, Iran

ABSTRACT

Background and Objectives: The most prevalent malignancy among women is known to be breast cancer (BC). Several factors contribute to determining tumor prognosis and treatment strategies. In this study, the frequency and relevance of these factors are discussed.

Materials and Methods: This cross-sectional study was carried out on 214 patients with BC, who referred to the Cancer Institute of Imam Hospital complex, Tehran, Iran in 2010 and 2011. The data about biomarkers (ER, PR, P53, HER-2) status and clinic pathologic features were extracted from patients files.

Results: Invasive ductal carcinoma (90.7%) was the most common pathology of BC. The frequency of estrogen receptor (ER), progesterone receptor (PR), P53 and HER-2 was estimated as 63.6%, 58.9%, 37.4% and 21.9% respectively. None of these markers had significant relationship with age, tumor size, tumor pathology, vascular invasion, calcification, nipple invasion, benign component, skin invasion and mitosis. Between low grade histology of tumor and ER, PR significant positive relationship was found ($P=0.001$). Lymph node involvement was positively associated with P53 expression. A positive relationship found between ER and PR ($P=0.001$). Both P53 and HER-2 inversely correlate with ER, PR ($P=0.001$). HER-2 and P53 had no significant relationship.

Conclusion: We found a significantly higher PR⁽⁺⁾, ER⁽⁺⁾ expression in low grade tumors. Although P53 and HER-2 expressions were not found to be correlated with tumor grade, P53 expression was associated with poorer prognosis due to higher lymph node involvement and perineural invasion.

Keywords: Breast Cancer, Estrogene Receptores, Progestrone Receptors, HER-2

Received: 21 April 2012

Accepted: 25 September 2012

Address communications to: Dr Alireza Abdollahi, Department of Pathology, Imam Khomeini Teaching Hospital, Tehran University of Medical Sciences, Tehran, Iran

Email: dr_p_abdollahi@yahoo.com

Introduction

Breast cancer (BC) is known to be the most prevalent malignancy among women (1), considering about 24.4% of all neoplasms in Iranian population. Incidence rate and characteristics of breast cancer was different regionally. In our country the lower incidence rate was reported, otherwise the average age of breast cancer diagnosis was lower (45-55 years) compared to western areas (reported as 50-60 years) (2).

The extremely lower prevalence of early stages in our country (1.02% compared 15-30%) (3), indicated that the vast majority of BC patients are diagnosed in advanced stages. Moreover, the life expectancy and quality of life was lower in Iranian patients compared patients in western countries.

On the other hand, current survey in our country asserts that the number of patients with newly diagnosed BC increased during recent decades (1).

Age, tumor pathology, lymph node involvement, tumor size and histological grading as well as some molecular features such as ER, PR, HER-2, P53 and Ki67, could influenced breast cancer prognosis (4-8).

According to prior studies, ER, PR positive patients seem to had a better prognosis both regarding survival rate and response to hormonal therapy (5), whereas HER-2 was introduced as a poor prognostic factor which correlates with some malignant features of tumors like instigation of cell proliferation, invasion and metastasis (9).

Immunohistochemistry (IHC) refers to the process of detecting antigens in cells of a tissue section by exploiting the principle of antibodies binding specifically to antigens in biological tissues (3, 4).

IHC is a common available method used to detect abnormal cellular phenomena (including malignant tumors, aberrant proliferation or cell death), localize the distribution of different biomarkers in tissues and determine biomarker status (4,10). To observe the interaction between antibodies and their receptive antigens we can use a variety of methods, including the application of color-producing reactions. In addition, this can be done by tagging the antibody with fluorescent molecules such as fluorescein or rhodamine (10).

Biomarker statuses besides clinicopathological factors were effective in determining BC prognosis. Therein these factors could be affected by demographic and ethnical situations. In this study we aimed to assess the relationship between different biomarker expression and clinicopathological features in Iranian patients with BC. Studies like this can help in making decision in treatment and also help to reduce treatment costs.

Materials and Methods

This cross-sectional study was performed on 214 breast cancer patients, who referred to Cancer Institute, Imam Hospital Complex, affiliated to Tehran University of Medical Sciences (TUMS) from September 2010 to September 2011. All samples diagnosed with breast cancer and undergone immunohistochemistry, were included in the study without any restrictions. The data about biomarkers (ER, PR, P53, HER-2) status and clinicopathologic features (age, tumor size, histological type, histological grade and lymph node involvement) were extracted from patients files.

In IHC laboratory of cancer institute, the samples are stained as follows: Initially the

samples are placed in paraffin blocks, then discharged and incubated with hydrogen peroxide 3%. Antigen retrieval in citrate buffer 1% with $\text{pH}=6$ for 20 minutes in microwave is done. In the next step, cuts are incubated with own specific antibody made by Dako Company (Denmark). For the next stage streptavidin-peroxidase is used and finally Di amino benzidine tetra hydro chloride is applied. In this laboratory Bloom-Richardson scoring is used for HER-2 scoring as well as ER and PR more than 1% is considered positive.

The SPSS 16 package for windows (Chicago, Illinois, USA) was used for analysis. Difference in Qualitative parameters compared using chi square (X^2) and fisher's exact tests. Strength of the correlation between our parameters was assessed using the Pearson spearman correlation test. Statistical level of significant considered as P value < 0.05 .

Results

Invasive ductal carcinoma (90.7%) was the most common pathology of breast cancer in our study. Others included invasive lobular carcinoma (4.2%), ductal carcinoma insitu (3.3%) and lobular carcinoma insitu (0.9%). The mean age of patients estimated 50.2 ± 12.3 years; patients aged 45 to 50 years were the most frequent age group. The mean tumor size and average number of lymph node involvement was estimated 2.95 ± 2.17 cm and 3.3 ± 3.3 ; respectively.

Table 1 showed the frequency of different prognostic factors among breast cancer patients. We assessed the frequency and the correlations between different biomarkers expressions and clinic pathological characteristics of BC.

Table 1- Frequency of prognostic factors in breast cancer

	Variable	Number (%)
Age (yr)	<50	103 (49)
	≥ 50	111 (51)
ER	Positive	136 (63.6)
	Negative	78 (36.4)
PR	Positive	126 (58.9)
	Negative	88 (41.1)
P53	Positive	80 (37.4)
	Negative	134 (62.6)
	0	164 (76.6)
HER-2	1+	3 (1.4)
	2+	6 (2.8)
	3+	40 (18.7)
Histology grade	I	19 (8.4)
	II	177 (82.7)
	III	18 (8.87)
Pathology	Invasive ductal carcinoma	194 (90.7)
	Other types	20 (9.3)
	Vascular invasion	
Positive		97 (45.3)
	Negative	117 (54.7)
Perineural invasion	Positive	48 (22.4)
	Negative	166 (77.6)

To determine the relationship between different hormonal receptor expressions we found below results; ER and PR were positively correlated with each other ($P < 0.001$). Both HER-2 and P53 expressions was inversely related to ER⁽⁺⁾, PR⁽⁺⁾ status ($P < 0.001$). No significant relation was found between P53 and HER-2 expression.

Patients categorized into two groups based on the tumor pathology; Group A (invasive ductal carcinoma) and group B (other types). No significant relationship was found between ER, PR, P53 and HER-2 expressions and the tumor pa-

thology in these groups. There was a significant correlation between P53 expression and lymph node involvement as well for perineural invasion (PV=0.004; $P=0.04$); this correlation was not observed in other biomarkers including ER, PR, HER-2. Histological grade I, II positively correlate with ER⁽⁺⁾ and PR⁽⁺⁾ status ($P<0.001$), however no relationship found between histological grade and P53, HER-2 expressions.

No significant relationship was observed between age and ER ($P=0.2$), PR ($P=0.6$), P53 ($P=0.41$), HER-2 ($P=0.41$) expression; Likewise there was no relation between tumor size and mentioned biomarkers ($P>0.05$).

Discussion

In this study, we have studied 214 patients whose average age was reported 50.2 years. This result is compatible with previous studies (4, 10). This resemblance could be due to similar lifestyle such as physical activity, diet, smoking, etc.

We found no relationship between age and IHC markers whereas Sirati reported a direct relationship between age and ER. Likewise, Taucher *et al.* (11) observed a significant correlation between HER-2 and lower age. The study by Huang (12) shows similar results with ours.

The most frequent pathology of BC in our study was invasive ductal carcinoma, which is compatible with Azizun-nisa and Taucher (4, 11) studies. Compatible with Azizun-nisa (4) study we found Grade II as the most frequent histological type of BC.

We found a significant correlation between low histological grade of tumor and PR⁽⁺⁾, ER⁽⁺⁾ status, this relation was not significant for P53, HER-2; these findings were similar to the results of other studies (4, 7, 12, 13).

In contrary to our findings, Rodrigues *et al.* (14), Nyaqol *et al.* (15) and Ivkovic Kapicl *et al.*

(16) represented that HER-2 was significantly correlated with histological grade III. Study conducted on 283 breast cancer patients with auxiliary lymph node involvement by Tokatli *et al.* declared a significant relation between HER-2, P53 expressions and lymph node involvement; in our study only P53 related to lymph node involvement.

The observed relationship between different hormonal receptor expressions in our study was fully compatible with Sirati *et al.* (10). The frequency of HER-2⁽⁺⁾, ER⁽⁺⁾, PR⁽⁺⁾ and P53⁽⁺⁾ was reported 21.9%, 63.6%, 58.9% and 37.4% respectively which was in accordance with some other studies (15,18,19). In our survey we did not find any relation between P53 and HER-2 expression which was incompatible with Fakheri *et al.* (20). In a study on 120 samples of invasive ductal carcinoma by Ivkovic-kapicl (16), 20% of samples were HER-2⁽⁺⁾ and a significant relation between HER-2⁽⁺⁾ and ER⁽⁻⁾, PR⁽⁻⁾ was reported which was in the same line of ours.

Another point regarding immunohistochemistry markers is their significant contribution to choosing a treatment strategy. Some treatment methods and medications like tamoxifen in hormonotherapy depend on the receptors' hormones. The way IHC is done also affect the results.

Conclusion

In our study, together with a positive correlation between ER and PR, they inversely correlated with P53 and HER-2. In addition, we found a significantly higher PR⁽⁺⁾, ER⁽⁺⁾ expression in low grade tumors. Although P53 and HER-2 expressions were not found to be correlated with tumor grade, P53 expression was associated with poorer prognosis due to higher lymph node involvement and perineural invasion.

Acknowledgment

This paper is the result of a thesis and was financially supported by Research Council of Tehran University of Medical Science. The authors declare that there is no conflict of interest.

References

1. Harirchi I, Kolahdoozan S, Karbakhsh M, Chegini N, Mohseni SM, Montazeri A, *et al.* Twenty years of breast cancer in Iran. *Ann Oncol* 2011;22(1):93-7.
2. Ministry of Health & Medical Education. Iranian annual of national cancer registry 2005-2006. Tehran:Tandis;2008.
3. Omranipour R, Fattahi A. Prevalence of ductal carcinoma insitu of the breast in Tehran University Medical Centers: evaluation of 2244 cases. *Tehran Univ Med J* 2009;67(7):516-21.
4. Azizun – Nisa, Bhurgri Y, Reza F, Kayani N. comparison of ER, PR and HER-2 reactivating pattern with histologic grade, Tumor size and lymphocytic status in breast cancer. *Asian pac J Cancer Prev* 2008;9(4):553-6.
5. Chen XS, Ma CD, Wu JY, Yang WT, Lu HF, Wu J, *et al.* Molecular subtype approximated by quantitative estrogen receptor, progesterone receptor and HER-2 can predict the prognosis of breast cancer. *Tumori* 2010;96(1):103-10.
6. Fredholm H, Eaker S, Frisell J, Holmberg L, Fredriksson I, Lindman H. Breast Cancer in Young Women: Poor Survival despite Intensive Treatment. *PLoS One* 2009;4(11):e7695.
7. Ambroise M, Ghosh M, Mallikarjuna VS, Kurian A. Immunohistochemical Profile of Breast Cancer Patients at a Tertiary Care Hospital in South India. *Asian Pac J Cancer Prev* 2011;12(3):625-9.
8. Pleșan DM, Georgescu CV, Pătrână N, Pleșan C, Stoica D. Immunohistochemical study of p53 and Ki67 in a group of patients with mammary carcinoma. *Rom J Morphol Embryol* 2010;51(3):459-65.
9. Hung HJ, Neven P, Drijckoningen M, Parideans R, WildiresH, Berteloot P, *et al.* Association between Tumor characteristics and HER-2 by immunohistochemistry in 1362 women with primary operable breast cancer. *J Clin Pathol* 2005;58(6):611-6.
10. Sirati F, Ghahri A. Detection of prognostic factors in breast cancer and their relation with menopause and age. *Iran J Surg* 2007;15(3):41-52.
11. Taucher S, Rudas M, Mader M R, Gnant M, Dubsy P, Roka S, *et al.* Do we need HER-2/neu Testing for all patients with primary breast carcinoma. *Cancer* 2003;98:2547-53.
12. Huang HJ, Neven P, Drijckoningen M, Paridaens R, Wildiers H, Van Limbergen E, *et al.* Hormone receptors do not predict the HER-2/neu status in all age groups of women with an operable breast cancer. *Ann Oncol* 2005;16(11):1755-61.
13. Huang HJ, Neven P, Drijckoningen M, Paridaens R, Wildiers H, Van Limbergen E, *et al.* Association between HER-2/neu and the progesterone receptor in oestrogen-dependent breast cancer is age-related. *Breast Cancer Res Treat* 2005;91(1):81-7.
14. Rodrigues NA, Dillon D, Carter D, Parisot N, Haffty BG. Differences in the pathologic and molecular features of intra ductal breast cancer between Younger and older women. *Cancer* 2003;97(6):1393–1403.
15. Nayqol J, Nyonqo A, Byakika B, Muchiri L, Cocco M, de Santi MM, *et al.* Routine assessment of hormonal receptor and HER-2/ neu status underscores the need for more therapeutic targets in Kenyan women with breast cancer. *Annal Quant Cyta Histol* 2006;28 (2):97–103.
16. Ivkovic-kapicel T, Knezevic –Usaj S, Djilas-Ivanovic, Panjkovic M. Correlation of HER-2/ neu protein over expression with other prognostic and predictive factors in invasive Ductal breast cancer. *Anti Cancer Research* 2007;21(4):673-8.
17. Tokatli F, Altaner S, Usal C, Ture M, Kocak Z, Uyqun K, *et al.* Association of HER-2 over expression with the number of involved axillary lymph nodes in human receptor positive breast cancer patients. *Exp Oncol* 2005;27(2):145-9.
18. Ariga R, Zarif A, Korasick J, Reddy V, Siziopikou K, Gattuso P. Correlation of HER-2/neu gen amplification

with other prognostic and predictive factors in female breast carcinoma. *Breast J* 2005;11(4):278–80.

19. Guarneri V, Giovannelli S, Ficarra G, Bettelli S, Moiorana A, Piacentini F, *et al.* Comparison of HER-2 and Hormone receptor expression in primary breast cancer and a synchronous paired metastases: impact on

patient management. *Oncologist* 2008;13(8) 838-44.

20. Fakhri T, Najafi B, Fadakar soogheh GH. Correlation of P53 Protein Expression with clinicopathologic parameters and Hormonal receptor status in breast carcinoma. *Guilan Uni Medical Sci J*2006;15(58):1-6.