RESEARCH ARTICLE

# Expressional Correlation of Human Epidermal Growth Factor Receptor 2, Estrogen/Progesterone Receptor and Protein 53 in Breast Cancer

**Marzieh Panahi[1], Najmaldin Saki[2], Sara Ashourzadeh[2], Fakher Rahim[3]***

## Abstract

**Background: This study aimed to show the localization of estrogen / progesterone receptors, human epidermal growth factor receptor 2 (Her-2) and protein 53 (p53) by immunohistochemistry in a series of consecutive breast cancer patients. Materials and Methods: The study covered invasive breast cancers from 299 patients presenting at the Oncogenetic Clinic and Pathology Centers of Ahwaz Jondishapour University of Medical Sciences Hospital in Iran during the time period from 2009 to 2011. The Scarff-Bloom Richardson scoring method was used. Results: Of the 299, 27% (80/299) were <40, 33% (100/299) were 41-50, and the remaining 40% (119/299) were>50 years old. The highest incidence of breast cancer in this study population was in the group of more than 50 year age, and the most common histological type of breast cancer was the invasive ductal carcinoma, which accounted for 68% (203/299) of the cases. Out of possible total of 207, 6% (13/207), 41% (85/207), and 53% (109/207) were scored as grade I, II, III, respectively. Conclusion: Our findings demonstrated a lack of association between labeling for the markers studied and tumor size and age of the patients. We confirmed an association between ER labeling and nuclear grade of breast cancer. The conflicting results obtained compared with the literature be because of differences in the immunohistochemical techniques applied in the various studies and to the scoring systems used.**

**Keywords:** Breast cancer - estrogen receptor (ER) - progesterone receptor (PR) - p53

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## Introduction

Breast cancer composes a remarkably diverse group of diseases regarding presentation, morphology, molecular profile and response to therapy. The risks of both breast cancer and death because of breast cancer are clearly increasing worldwide. Some 45% of the more than 1 million new cases of breast cancer diagnosed each year, and more than 55% of breast-cancer–related deaths, occur in low- and middle-income countries (Matsuda et al., 2013). Breast cancer affects Iranian women at least one decade younger than their counterparts in developed countries (Mousavi et al., 2007). For instance, in Iran it has been shown that, even after adjusting for age, young women are at higher risk for developing breast cancer than are their Western counterparts (Harirchi et al., 2010). The mortality rate of breast cancer was 5.8 per 100,000 women in Tehran in 1998 (Mohagheghi et al., 2010), 2.5 per 100,000 for female population, and 7762 years life lost in the 18 provinces of Iran in 2001 (Khosravi et al., 2007).

Breast cancer is not a single disease but a group of several important tumors subtypes, each with a different natural history and each requiring a different treatment. Key factors such as tumor size, histological grade, vascular invasion, and nodal status are helpful, but increasing attention is being paid to the molecular features of the tumor (Schonborn et al., 1994). Many investigations have been performed about these regulators considering the role of steroid hormone and growth factor receptors, to growth and differentiation of both normal and malignant human breast cells. The discovery of the biomarkers opened a new view to diagnosis and treatment of these diseases.

Many studies of gene expression have identified expression profiles and gene sets that are prognostic, predictive, or both for patients with breast cancer (van de Vijver et al., 2002; Sorlie et al., 2003; Chang et al., 2004; 2005; Ma et al., 2004; Paik et al., 2004; Bertucci et al., 2005; Wang et al., 2005; Habashy et al., 2011; Patsialou et al., 2012; Tian et al., 2013). Prognostic and predictive biomarkers for breast cancer commonly used in clinical practice include estrogen receptor (ER) and progesterone receptor (PR) over-expression, oncogene over-expression c-erbB2 , human epidermal growth factor receptor 2 (Her-

*[1]Department of Histology, School of Medicine, [2]Thalassemia and Hemoglobinopathies Research Center, [3]Toxicology Research Center, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran *For correspondence: Fakherraheem@yahoo.com*

2), protein 53 (p53), protein 21 (p21) and etc. Her-2 is a normal cellular gene that encodes a membrane protein 185 (P185) and its amplification plays an important role in the pathogenesis of breast cancer (Esteva-Lorenzo et al., 1998; Szoke and Udvarhelyi, 2012).

Her-2/neu over-expression tumors were shown to increase disease recurrence and metastasis and shorten survival (Cho et al., 2008; Szoke and Udvarhelyi, 2012; Dairkee et al., 2013). p53 is a tumor suppressor gene and an important component of breast cancer pathophysiology (Cho et al., 2008). The intensity of estrogene receptor (ER) expression in normal epithelium risk is a risk factor for breast cancer conferring a 3-fold increase in risk (Dairkee et al., 2013). Similarly to ER, PR has been found elevated very early in pre-malignant breast lesions at the hyperplasic enlarged lobular unit (Lagiou et al., 2009). In regarding to follow up difficulties cases in developing countries that lack screening programmes (as in Iran), we have studied localization of ER/PR, Her-2 and p53 by Immuno-histochemistry in consecutive breast cancer specimens submitted to pathology centers, and compared the labeling for these markers with histological type and grade of the cancers as well as type of breast cancer, age and size of tumor of these patients.

## Materials and Methods

*Patients*

Patients with breast cancer and who had a family history of breast or ovarian cancer, or both, that was compatible with a dominant mode of inheritance were selected. We evaluated invasive breast cancers from 299 patients during time period from 2010 to 2013. These patients were asked to provide a blood sample and to sign an informed-consent form authorizing an analysis for analysis of molecular biomarkers. All procedures were approved by international guidelines and by the Institute Research Ethics and Use Committee of Ahwaz Jondishapour University of Medical Sciences (AJUMS). The samples were fixed in 10% formaldehyde solution. The tissue were processed routinely for embedding in paraffin wax and 5$\mu$m thick sections were cut and placed on glass slides coated with 3-Aminopropyl Triethoxy Silane (APES) to enhance adhesion of sections to the slides for immuno-histochemistry. One slide of each tissue was stained with Hematoxylin and Eosin (H&E) to determine the histological type and grade of tumor.

*Immunohistochemistry (IHC)*

Briefly, 5-micron sections were cut, deparaffinized in xylene, rehydrated in a series of graded alcohols and placed in a tris buffer bath. Endogenous peroxidase activity was quenched using 3% hydrogen peroxide. Slides were rinsed with deionized water and placed in a tris buffer bath. After incubation, 1% preimmune goat serum was used to block nonspecific staining, and sections were stained with primary antibodies, respectively. Biotinylated link antibodies were added using the Labeled Streptavidin-biotin (LSAB) Kit (Dako), according to manufacturer's instructions. Detection was achieved using Diaminobenzidine (DAB) and $H_2O_2$ as substrates. All used antibodies were purchased from Dako. The following list includes all antibodies: c-erbB-2, rabbit antihuman c-erbB-2 oncoprotein; p53, mouse monoclonal antihuman p53; ER, mouse monoclonal antihuman ER; and PR, mouse monoclonal antihuman PR. Expression of ER/PR and p53 was graded as weakly positive (<10% the cells are stained), and positive when more than 10% tumor cell's nucleus stained, whereas absences of staining were considered as negative.

We have used the Scarff-Bloom Richardson scoring method (Eleston and Ellis, 2002). In short, the Scoring criteria for Her-2/neu were as follows: 1, Zero score defined tumors with no staining; 2, 1+ score refers to membrane staining (not continually) in less than 10% of tumor cells; 3, 2+ score which is characterized by weak to moderate complete membrane staining in more than 10% of the tumor cells; 4, 3+ score is defined as strong complete membrane staining in more than 10% of the tumor cells (high intensity). If the tumor was 0 or 1+, it was considered Her-2 negative and if the tumor was 2+ or 3+ Her-2 was positive. Tumor size was categorized macroscopically in to three classes, <2, 2-5 and >5 cm, respectively.

*Statistical analysis*

Statistical analysis was performed using SPSS version 13.0. Relationships between tumor markers and other parameters were studied using the test. Differences at $p<0.05$ were considered as statistically significant.

## Results

Two hundred ninety nine (299) breast cancer women were included in this study; all of the samples had embedded in paraffin blocks. Of these 299, 27% (80/299) were <40, 33% (100/299) were 41-50, and the rest 40% (119/299) had >50 years old. The highest incidence of breast cancer in this study population was in the group of more than 50-year age and the most common histological type of breast cancer was the invasive ductal carcinoma, which accounted for 68% (203/299) of the cases. The tumor grades were performed only in 207 patients and for the rest grading was out of rule. Out of total 207, 6% (13/207), 41% (85/207), and 53% (109/207) were scored as grade I, II, III, respectively. IHC staining of four biomarkers (Her-2, ER, PR and p53) were performed for all patients. Relationship between different marker labeling and various known prognostic markers are

**Table 1. Relationships between Marker Labeling and Tape of Breast Cancer**

| Cancer Type (No. of cases) | Prognostic Markers (No. (%)) | | | |
|---|---|---|---|---|
| | G1 | G2 | G3 | G4 |
| Invasive ductal carcinoma (203) | 76 (37.50) | 104 (51.23) | 100 (49.3) | 101 (49.3) |
| Inflamatory ductal carcinoma (45) | 28 (62.20) | 29 (64.50) | 23 (51.1) | 26 (57.8) |
| Insitu ductal carcinoma (38) | 15 (39.47) | 17 (44.70) | 19 (50.0) | 16 (41.1) |
| Medulary carcinoma (13) | 10 (76.92) | 8 (61.53) | 12 (92.3) | 11 (84.6) |
| Total (299) | 137 (45.81) | 158 (52.84) | 159 (53.2) | 140 (46.8) |
| P-value | 0.006 | 0.022 | 0.034 | 0.042 |

*Abbreviation: G1) ER-positive and/or PR-positive and HER2-negative; G2) HER2-positive and ER-positive: G3) HER2-positive and ER-negative; and G4) Triple negative (negative for ER, PR and HER2)

**Table 2. Relationships between Marker Labeling and Grade of Breast Cancer**

| Grade (No. of cases) | Markers | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | G1 | | G2 | | G3 | | G4 | |
| | + | - | + | - | + | - | + | - |
| I (13) | 11(84.6) | 2(15.4) | 9(69.2) | 4(30.8) | 4(30.8) | 9(69.2) | 6(46.2) | 7(53.8) |
| II (80) | 47(58.8) | 33(41.2) | 43(53.8) | 37(46.2) | 39(48.8) | 41(51.2) | 43(53.8) | 37(46.2) |
| III (109) | 65(60.0) | 44(40.0) | 54(50.4) | 55(50.6) | 57(52.0) | 52(48.0) | 52(48.0) | 57(52.0) |
| Total (202) | 123(60.9) | 79(39.1) | 106(52.5) | 96(47.5) | 100(49.5) | 102(51.5) | 101(50.0) | 101(50.0) |
| P-value | 0.006 | | 0.022 | | 0.034 | | 0.042 | |

*Abbreviation: G1) ER-positive and/or PR-positive and HER2-negative; G2) HER2-positive and ER-positive: G3) HER2-positive and ER-negative; and G4) Triple negative (negative for ER, PR and HER2)

**Table 3. Relationships between marker Labeling and Size of Breast Cancer**

| Size (No. of cases) | Markers | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | G1 | | G2 | | G3 | | G4 | |
| | + | - | + | - | + | - | + | - |
| Less than 2 cm (34) | 21(61.8) | 13(38.2) | 21(61.8) | 13(38.2) | 13(38.2) | 21(61.8) | 20(58.8) | 14(41.2) |
| 2-5 cm (169) | 104(61.5) | 65(38.5) | 86(50.9) | 83(49.1) | 90(53.3) | 79(46.7) | 84(49.7) | 85(50.3) |
| More than 5 cm (10) | 6(60.0) | 4(40.0) | 5(50.0) | 5(50.0) | 5(50.0) | 5(50.0) | 3(30.0) | 7(70.0) |
| Total (213) | 131(61.5) | 82(38.5) | 112(52.6) | 101(47.4) | 108(50.7) | 105(49.3) | 107(50.2) | 106(49.8) |
| P-value | 0.995 | | 0.264 | | 0.504 | | 0.279 | |

*Abbreviation: G1) ER-positive and/or PR-positive and HER2-negative; G2) HER2-positive and ER-positive: G3) HER2-positive and ER-negative; and G4) Triple negative (negative for ER, PR and HER2)

**Table 4. Relationships between Marker Labeling and Age**

| Age Groups (No. of cases) | Markers | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | G1 | | G2 | | G3 | | G4 | |
| | + | - | + | - | + | - | + | - |
| <40 (67) | 32(47.8) | 35(52.2) | 30(44.8) | 37(55.2) | 40(59.7) | 27(40.3) | 36(53.80) | 31(46.2) |
| 40-50 (113) | 77(68.2) | 36(31.8) | 71(62.8) | 42(37.2) | 55(48.7) | 58(51.3) | 55 (48) | 58(52.0) |
| >50 (119) | 71(59.7) | 48(40.3) | 58(48.7) | 61(51.3) | 62(52.1) | 57(47.9) | 61(52.11) | 58(47.9) |
| Total (299) | 180(60.2) | 119(39.8) | 159(53.2) | 140(46.8) | 157(52.5) | 142(47.5) | 152(50.80) | 147(49.1) |
| P-value | 0.025 | | 0.029 | | 0.356 | | 0.801 | |

*Abbreviation: G1) ER-positive and/or PR-positive and HER2-negative; G2) HER2-positive and ER-positive: G3) HER2-positive and ER-negative; and G4) Triple negative (negative for ER, PR and HER2)

**Table 5. Relationships between Marker Labeling and Nuclear Grade of Breast Cancer**

| Grade (No. of cases) | Markers | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | G1 | | G2 | | G3 | | G4 | |
| | + | - | + | - | + | - | + | - |
| I (17) | 17(100) | 0(0.00) | 15(88.2) | 2(11.8) | 7(41.2) | 10(58.8) | 11(64.7) | 6(35.3) |
| II (138) | 81(58.7) | 57(41.3) | 68(49.3) | 70(50.7) | 66(47.8) | 72(52.2) | 68(49.3) | 70(50.7) |
| III (46) | 21(45.7) | 25(54.3) | 19(41.3) | 27(58.7) | 29(63.0) | 17(37.0) | 26(56.5) | 20(43.5) |
| Total (201) | 119(59.2) | 82(40.8) | 102(50.7) | 99(49.3) | 102(50.7) | 99(49.3) | 105(52.2) | 96(47.8) |
| P-value | 0.00049 | | 0.003 | | 0.144 | | 0.395 | |

*Abbreviation: G1) ER-positive and/or PR-positive and HER2-negative; G2) HER2-positive and ER-positive: G3) HER2-positive and ER-negative; and G4) Triple negative (negative for ER, PR and HER2)

summarized in Tables 1-5.

A significant relationship ($p<0.25$) was demonstrated between the used biomarkers and the type of breast cancer. The results also showed that there is a significant relationship ($p<0.006$) among ER labeling and invasive and inflammatory ductal carcinoma. None of the biomarkers were demonstrated a significant relationship with Insitu ductal carcinoma but they have lonely a significant relationship ($p<0.006$) with medulary carcinoma (Table 1). All labeling markers were showed no significant correlation with any of grades (Table 2). All four biomarkers didn't have any significant relationship with tumor size and age of patients except ER and PR labeling had a significant correlations with 41-50 years ($p<0.073$) old and >50 old of ages ($p<0.096$)

## Discussion

Nowadays molecular markers seem to have the potential improvement on our capacity to taking care of patients with, or at risk for breast cancer. In this study, we have compared labeling of four markers with some of known prognostic factors including histological type of cancer, histological grade, and nuclear grade, size of tumor and age of patients. In regarding to results each of biomarkers lonely has a significant association with the type of breast cancer. ER is expressed in about 70% of invasive breast cancer (Lee et al., 2007) and in our study ER labeling showed a significant relationship with invasive ductal carcinoma and medullary carcinoma. Inflammatory breast cancer is rare type of invasive

breast cancer accounts for about 1% of all breast cancers; therefore, differentiated duct carcinomas of breast express ER and are generally, responsive to hormone therapy (Yenidunya et al., 2011; Fasching et al., 2012). PR expression commonly parallels that of ER expression, which is confirmed by the strong correlation between the labeling of the two receptors in the present study and other studies (Deblois and Giguère, 2013).

A number of clinical studies have documented an association between HER-2 amplification/overexpression and negative steroid hormone receptors (HR) status in breast tumors (Rosenthal et al., 2002; Muller et al., 2003; Jehoram et al., 2005; Ellis et al., 2006; Rody et al., 2009). In general, the higher the level of HER-2 overexpression and gene amplification will show the lower corresponding ER level. Our data also demonstrate an inverse correlation between HER-2 protein/gene levels and PR levels that most likely occurs because suppression of ER expression leads to reduced expression of PR. The negative correlation between HER-2 labeling and PR labeling supports the findings of others that HER-2 expression is a marker of poor prognosis.

The results of many studies suggest the p53 expression, HER-2 expression, and coexpressions of HER-2 and p53 have prognostic significance in breast cancer. Over-expression of HER-2 correlated strongly with poor patient survival (Ouyang et al., 2001; Yang et al., 2012) but contrasts with studies that suggest HER-2 over-expression has no (Erdem et al., 2005) or only limited prognostic value (Barnes et al., 1988). Coexpression of HER-2 and p53 has been reported in several studies, with frequency of coexpression as high as 42% (Rudas et al., 1997; Thor et al., 1998; Umekita et al., 2000; Kazkayasi et al., 2001; Bull et al., 2004; Skálová et al., 2009). Patients whose breast cancer tissues express HER-2 and p53 have been found to have a poor prognosis in several studies (Tsuda, 2009).

In conclusion, these conflicting results may be because of difference in the immunohistochemical techniques applied in the various studies and to the scoring systems used. Our findings also confirm lack of association between labeling for the markers studied and tumor size and age of the patients. We have confirmed the association between ER labeling and nuclear grade of breast cancer.

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