Original Article

Correlation of ER, PR and HER-2/Neu with other Prognostic Factors in Infiltrating Ductal Carcinoma of Breast

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ABSTRACT

Background and Objectives: Breast cancer is the most common malignancy among women in the world. The aim of this study was to assess estrogen receptor (ER), progesterone receptor (PR) and HER-2/neu of infiltrating ductal carcinoma (IDC) with tumor size, histologic grade, lymph node metastasis and age.

Methods: This study was carried out on 300 tissue blocks ofpatients with IDC who underwent mastectomyfrom 2007 to 2011 in ShahidBeheshti Hospital, affiliated to Babol University of Medical Sciences, Babol, Iran. Data including age, tumor size, and histologic grade and lymph node status retrieved from pathology department.

Result: Themean age of the patients was 40.2±2.3 (ranged 19-82 years). ER and PR were positively correlated with each other (*P*= 0.001) and they inversely correlated with HER-2/neu(*P*=0.001). We observed correlation between ER and PR expression and low histologic grade (*P*= 0.001) and HER-2/neu expression and high histologic grade (*P*= 0.003). There was correlation between HER-2/neu expression and lymph node involvement (*P*=0.03). None of these makers showed correlation with age and tumor size (*P*> 0.05).

Conclusion: Our findings indicate the importance of ER, PR and HER-2/neu expression asprognostic factors for therapeutic decision.

Keywords: Estrogen Receptors, Progesterone Receptor,c-erbB-2 Proto-Oncogene, Invasive Ductal Carcinoma, Breast

Introduction

Breast cancer is the most common malignancy and the leading cause of cancer related death among women all around the world (1,2). In Iran, it is the most common cancer among women and affects them one decade earlier than women in western countries (3,4). Besides, the prevalence of early stages among Iranian women is extremely low (5).

Factors including tumors size, histologic grade, lymph node metastasis and hormone receptor status used to estimate prognosis (6-8). Evaluation of prognostic factors including biomackers is highly recommended for the best management and therapeutic decision of breast cancer patients (9).

Among these biomarkers, estrogen receptor (ER), progesterone receptor (PR), and HER-2 / neuare mostly used for assessment of breast cancer. Her-2/neuprotooncogen (erbB-2) encodes a 185 kDa transmembranephosphoglycoprotein with tyrosine kinase activity. It is standard practice to determine both estrogen and progesterone receptor status at the time of diagnosis for definitive surgical therapy (10). HER-2/neu encodes a tyrosine kinase related to epidermal growth factor. Several studies revealedHER-2/neu overexpression as an indicator for low response to tamoxifen and decreased survival (8,10,11). Tumors with negative immunohistochemistry (IHC) staining for ER,PR and HER-2/neu were high grade and mostly seen in women older than 50 (12).

This study was performed to assess the possible correlation between expression of ER,PR and HER-2/neuwith other prognostic factors like tumor size, histologic grade, lymph node metastasis and age.

Materials and Methods

The present cross-sectional study was conducted on 300 paraffin blocks of patients, who underwent mastectomy and diagnosed with infiltrating ductal carcinoma (IDC) in ShahidBeheshti Hospital, Babol, northern Iran from 2007to 2012. Data including age,tumor size andgrade and lymph node status were retrieved from the files of patients in pathology department.

Patients were divided into two age groups: 1) < 50 years and 2) ≥50 years. Furthermore, tumor size was divided to 1) ≤2 cm and 2)>2cm. Bloom-Richardson scoring was used for histologic grading (grade I, II and III).

In brief, paraffin- embedded blocks were used to prepare 3 μm thick slides, then sections were deparaffinized in xylene and rehydrated through graded concentration of ethanol.For antigen retrieval, the slides were incubated with Tris-EDTA (PH=9) for 15 minutes in microwave. After washing with Tris-buffer saline (TBS) and tap water,all slides were incubated in H_2O_2-methanol solution (1/9) for 10 minutes to inhibit endogenous proxidases. At the next step, the slides were covered by primary antibody (Dako, Denmark) for 30 minutes at room temperature, followed by incubation with Envision. Then, all slides were covered by chromogen and Di amino Benzidine. ER,PR and HER2/neu were semi-quantitatively evaluated. H score was used for ER and PR (a negative result was defined as a score of ≤ 50, and positive between 51 and 300). The DAKO scoring system for HER-2/neu was defined as negative for scores of 0,1+, or 2+ and positive for tumors with a score of 3+(10,11) (Fig. 1-3).

Quantitative values of the data were presented as mean ±SD. A Chi-square test and *t*-test were used to determine statistical analysis using SPSS software version 20 for windows (Chicago,Illinois, USA) . A *P*-value of < 0.05 was considered as significant level.
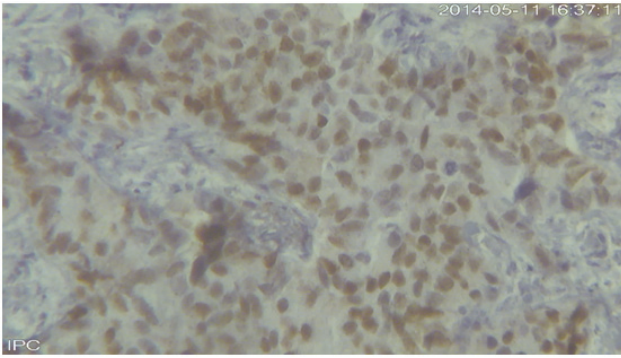
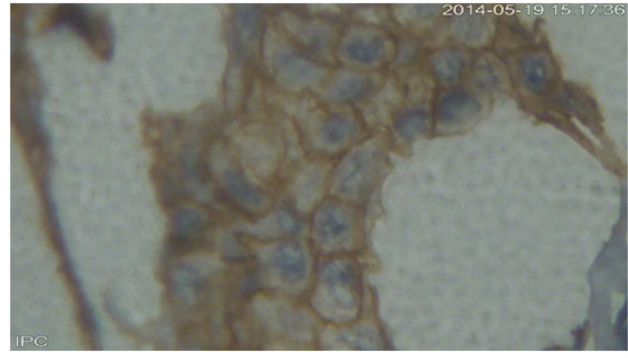**Fig.1:** Immunohistochemical staining showed positivity for ER

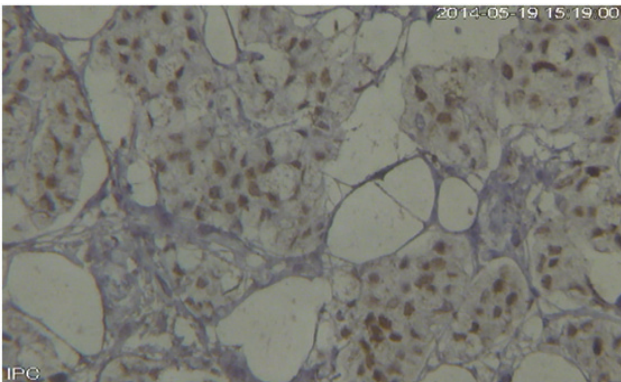**Fig.2:** Immunohistochemical staining showed positivity for HER2/neu

Fig.3: Immunohistochemical staining showed positivity for PR

ER and PR expression and histologic grade I and II (P= 0.001). On the other hand, significant correlation between HER-2/neu expression and histologic grade III was found (P= 0.003). The expression of these biomarkers had no significant correlation with age and tumor size (P>0.05).

Table 1: Clinical characteristics (n= 300)

Variable	N	%
Age(years)		
<50	220	73.33
≥50	80	26.66
Tumor size(cm)		
≤2	125	41.66
>2	175	58.33
Tumor grade		
I	28	9.33
II	242	80.66
III	30	10.00
Lymph node		
Negative	43	14.33
positive	257	85.66

Results

This study was performed on 300 paraffin tissue blocks of patients with IDC. The mean age of patients was 40.2±2.3 with range of 19 to 82 years. Patients aged 35 to 45 years were the most frequent age group, 102 cases (34%). Most of tumors, 242 cases (80.6%) belonged to histologic grade II. The tumor size ranged from 0.5 cm to 16 cm with a mean range of 3.15±2.52 cm. The average of lymph node involvement was 4.5±3.4. Of the 300 patients 85.7% (n= 257) had lymph node metastasis. Table 1 reveals the clinical characteristics of all 300 women with IDC.

ER and PR were positively correlated with each other (P=0.001). HER-2/neu was inversely related to ER and PR expression (P=0.0001). We demonstrated statistically significant correlation between HER-2/neu expression and lymph node metastasis (P=0.03). However, this correlation was not found for ER and PR. Statistically significant correlation was observed between

Discussion

The present study was conducted on 300 patients with IDC. In Iran, this is the first time that one investigates the correlation between ER,PR and HER-2/neu with some prognostic factors on large population of patients with IDC.

In the current study, the mean age of the patients was 40.2±2.3 that was lower than other studies from Iran (1,7,13). It shows that in the recent

years youngerwomen have been diagnosed with breast cancer. This fact may be explained by different factors that simply go beyond the scope of this study and must be investigated in future.

We observed a significant correlation between histologic grade I and II and expression of ER and PR that is similar to the results of other studies (7,12). Our study, as well as others, found a significant correlation between HER-2/neu expression and histologic grade III (10,14,15). Her-2/neu is an epidermal growth factor on the surface of a cell that transmits growth signals to the cell nucleus. Overexpression of the receptor is associated with poor prognosis. This may be more of a reflection of the biologic correlates of Her-2/neu overexpression that an independent prognostic indicator. Regarding sample size it seems that HER-2-neu expression is associated with poor prognosis while ER and PR expression are indicators of response to hormone therapy and better prognosis. Although hormone receptor status correlates with the prognosis, it does so to a lesser degree than nodal status. Hormone receptor determination is, however, of critical importance as a predictive factor. Therefore, it determines response or lack of response of a particular therapy.

In the present study, HER-2/neu expression was significantly correlated with lymph node involvement. This is in line with a study by Tokatli et al. (16). Lymph node status is important in determining cancer staging and treatment options. It is worth noting that it is the most important factor in the prognosis of patients with breast cancer. As the number of positive axillary lymph nodes increases, survival rate decreases and relapse rate increase.Many authors demonstrated HER-2/neu expression is associated with lymph node metastasis and found that number of positive lymph nodes and HER-2/neu expression were related to prognosis (17).Correlation between HER-2/neu expression and lymph node involvement and vascular invasion has already been observed. In addition,

the expression of HER-2/neu is an indicator of disease-free survival (16).

In contrary to other studies we found no correlation between tumor size and these biomakers (14,18,19). However, our result was similar with Ariga et al.findings (10). The exact explanation for these variable results is not known, eventhough tumor size is one of the prognostic factors.Besides, we did not observe correlation with age that was similar with previous studies (10,14). A study by Huang et al. showed that ER,PR and HER-2/neu expression varied with age. ER and PR status was not predicator of the HER-2/neu status in young age. However, in older women the hormone receptor status was associated with HER-2/neu (20).

In the present study, HER-2/neuexpression was significantly associated with ER and PR negative status (P= 0.0001). This is similar to study by Ariga et al. (10). It has been suggestedthat this association may reflect a better prognosis. However, other studies revealed ER-positive, HER-2/neu-positive status accompanied by poorer survival than ER-positive, HER-2/neu-negative status (21, 22). Therefore, it seems that HER-2/neu expression is a better predicator of response to hormonal therapy than ER status alone.

Study limitations include the fact that we investigated the biomarkers status only in IDC but not in other types of breast carcinoma.

Conclusion

Our results showed positive correlation between ER and PR accompanied by inverse correlation of them with HER-2/neu. In addition, we found significant correlation between ER and PR positive status and low grade tumors. On the other hand, correlation between HER-2/neu and high grade tumor and lymph node involvement was significant. These findings showed the importance of these biomarkers because they provide valuable prognostic information for best therapeutic decision.

Acknowledgments

Conflict of interest

The authors declare that there is no conflict of interests.

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