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HER-2, P53 and Hormonal Receptors Protein Expression as Predictive Factors in Breast Cancer Prognosis

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OBJECTIVE Breast cancer is a heterogeneous disease with variable biological and clinical characteristics. We conducted a study to evaluate P53, HER-2/neu and hormonal receptor expression as predictors of prognosis in breast cancer.
METHODS In a prospective study, we recruited 81 consecutive patients with primary operable breast cancer who were treated with mastectomy followed by locoregional radiotherapy or chemotherapy and studied the presence of P53, HER-2/neu and hormonal receptors (ER/PR) expression in tumor tissues by immunohistochemical staining. Associations between these markers expression and clinical outcomes, including local and regional recurrence and metastasis were evaluated. Statistical analysis was performed with the SPSS software.
RESULTS The mean time of follow-up was (47.3 ± 4.6) months. Expression of P53, HER-2/neu, Estrogen receptors and progesterone receptors were observed in 31.1%, 38.5%, 31.8% and 51.7% of the patients, respectively. P53, HER-2/neu and Negative ER status were potent predictors of local-regional recurrence (P = 0.034, 0.038, 0.044, respectively). Also HER-2/neu, Negative ER and Negative PR status were strong predictors of metastasis (P = 0.001, 0.042, 0.054, respectively).
CONCLUSION P53 and HER-2/neu expression and also steroid receptors status (ER/PR status) have an important role in predicting the outcome of breast cancer and thus may be of value in selecting suitable therapeutic strategy and determining prognosis in these patients.

KEY WORDS: breast cancer, HER-2/neu, P53.

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, clinical and pathological features, and outcome of treatment has been widely reported[1-3]. 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 Western Countries[4-9]. For instance, in Iran it has been shown that, even after adjusting for age, young women are at relatively higher risk for developing breast cancer than those counterparts in Western Countries[10].

There is no doubt that the lack of early detection program and awareness contribute to extension of disease, however, the biologi-

cal aggressiveness in terms of poor differentiation, lack of steroid receptor expression, and tendency to affect younger females remain unexplained. Abnormalities described in the structure and activity of several protooncogenes may contribute to the development or progression of primary breast carcinoma and its local-regional recurrence (LR), or metastasis[11-13]. Alterations in the protooncogenes p53 and HER-2/neu have been extensively studied in Western and East Asian females with breast cancer and proposed as prognostic markers of potential clinical utility[14,15]. However, few studies exist on prognostic significance of these oncogenes in female breast cancer in developing countries particularly in Iran[16-21]. Thus study of these markers and their impact on breast cancer prognosis in Iranian women is important and might contribute to the current knowledge on this important topic. The aim of the present study was to determine the impression of P53, HER-2/neu and hormonal receptors protein as predictors of local recurrence and metastasis after primary breast cancer tumor in Iranian women.

Materials and Methods

Patient selection

Our study was descriptive-analytical. The patients were chosen consecutively from the patients with primary breast cancer referred to our outpatient clinic as well as the private practice patients from 1996~2006 (a 10-year review). Census sampling was performed and sample size was 81 patients. All patients were thoroughly examined, received treatment and were followed up for the signs of recurrence and metastasis based on the physical examination, performance status assessment, chest X-ray, mammography, ultrasonography and serum tumor markers. The histopathological findings were based on simple reading of the "histopathological reports". The primary outcomes for this study were local regional recurrence (LR) and metastasis.

Data collection and statistical analysis

The necessary information was gathered and recorded in the questionnaires. Statistical analysis was performed with the SPSS statistical package (SPSS Ver14.0). All Continuous variables are expressed as mean ± SD, and dichotomous variables as frequencies. Categorical variables were compared using the chi-square test and continuous variables by using Student's *t* test and *P* values < 0.05 were considered statistically significant.

Results

The follow-up period ranged from 1 to 168 months (mean follow-up time was 47.3± 4.6 months) and 89.5% of patients were followed for more than 6 months. Patients and tumor characteristics have been listed in Table 1. The mean age of all patients was (48.30 ± 1.27) years (ranging from 21 to 84). A total of 81 patients underwent surgery. Chemotherapy was used in 60 patients (81.1%) and radiotherapy was administrated in 70 patients (93.3%). Local regional recurrence (LR) was noted in 4 patients (4.9%) and metastasis was seen in 10 patients (12.3%). P53 and HER-2/neu oncogene expression were detected in 31.1% and 38.5% of cases, respectively, also lack of estrogen and progesterone receptors expression were seen in 31.8% and 51.7% of patients, respectively.

There was significant correlation between age and local/regional recurrence (LR). Younger woman were at higher risk of recurrence (38.71 ± 4.39 *vs.* 49.8 ± 1.6; $P = 0.024$). Compared with free recurrence patients, in the local recurrence patient the age of first pregnancy was significantly lower (17.40 ± 1.14 *vs.* 21.31 ± 5.73; $P < 0.001$). Neither age nor the age at first pregnancy were correlated with metastasis, also there were no significant correlations between positive family history of breast cancer and recurrence or metastasis.

Expression of P53 immunopositivity significantly correlated to local/regional recurrence ($P = 0.034$) (Table 1), but it was not correlated to metastasis ($P = 0.22$). There were significant associations between HER-2/neu immunopositivity and local regional recurrence ($P = 0.038$) and metastasis ($P = 0.001$) (Table 1). Negative ER status was significantly correlated to local-regional recurrence ($P = 0.044$) and metastasis ($P = 0.042$) (Table 1), but negative PR status alone was correlated to metastasis ($P = 0.054$) and no association was found between negative PR status and local-regional recurrence ($P = 0.31$) (Table 1).

Discussion

Breast cancer has a major impact on women's health[22] Breast cancer is the most common female-related cancer that leads to death mostly in 40~45-year-old women[23]. The age-adjusted incidence of the new case has been steadily increasing since the middle of 1940s. In the 1970s' the probability of a woman in the United States developing breast cancer was estimated at 1 in 13. In 1980, it was 1 in 11, and in 1996 the frequency was 1 in 8.9. Women with a history of invasive breast cancer are at risk of developing metastatic disease, most recurrences are detected within 5~10 years after the diagnosis, but late recurrences can also occur[24]. As screening programs identify more patients with earlier stage disease, and as the number of women diagnosed with insitu ductal carcinoma continuously rises, there will be even more women living with a personal history of breast cancer[22]. On the other hand all patients who have experienced recurrence or metastasis are not considered at the end stage[22] and although the median survival of women with metastatic breast cancer is in the range of 2 to 3 years, there is a great variability. Indeed,

Table1. Relationships of patient' characteristics and tissue based markers to local regional recurrence and metastasis.

	Local regional recurrence (LR)			Metastasis		
	Yes	No	*P*	Yes	No	*P*
Age (years)	38.71 ± 4.39	49.8 ± 1.6	0.024	44.75 ± 3.70	49.35 ± 1.65	0.32
Monarch Age (years)	13.5 ± 1.51	13.59 ± 1.45	0.88	13.14 ±1.67	13.66 ± 1.40	0.37
First Pregnancy Age (years)	17.40 ± 1.14	21.31 ± 5.73	< 0.001	21.60 ± 5.41	21.10 ± 5.71	0.85
Breast feeding Duration (months)	15.2 ± 3.59	16.55 ± 1.32	0.73	17.80 ± 4.60	16.37 ± 8.31	0.71
Positive Family History (%)	12.5	12.5	0.89	12.5	12.5	0.89
ER Negative	75	26.9	0.044	62.5	26.5	0.042
PR Negative	75	48.9	0.31	85.7	46.7	0.054
Positive P53	75	24.3	0.034	50	25.7	0.22
Positive HER-2/neu	75	24.1	0.038	80	20	0.001

a small number of patients with metastatic disease who receive a course of chemotherapy remain relapse-free for a decade or longer[25]. Although tumor markers are known as indices for managing and following-up of breast cancer[23,24,26-30] two points are still being debated: i.e. their cost effectiveness has neither been demonstrated nor disproved; and the reliability of the currently used dichotomous division into positive/negative cut-off should be definitely valid[31]. Several studies have been done to define the sensitivity of these tests for detecting recurrence and metastasis. This study examines the prognostic significance of P53, HER-2/neu and ER/PR status in invasive breast cancer in Iran.

Abnormalities of the P53 gene and protein expression, which are usually associated with an allelic loss on chromosome 17, have been widely reported in breast carcinoma with variable mutation rate ranging between 40%~80%[11,26]. Significant association between P53 alterations and tumor size, histologic and nuclear grade, DNA ploidy, mitotic rate, proliferation index, positive node status, distant metastases, and lack of estrogen receptors were reported in white European and American, Afro-American, and East Asian females[1,15,32]. Just like previous studies, in our study P53 immunopositivity expression was seen in 31.1% of patients with statistically significant correlation between P53 immunopositivity and local/regional recurrence (Table 1), but contrary to Al-Moundhri M et al study[15], we did not find association between P53 expression and metastasis.

HER-2, which is also known as c-erbB-2 or neu, encodes a transmembrane protein belonging to the epidermal growth factor receptor family[33]. The HER-2 gene is either amplified or over expressed in 15%~30% of all invasive breast cancers. Amplification of the HER-2 gene means that instead of having only 2 copies of the gene per cell, there may be 50~100 gene copies per cell. The number of HER-2 proteins per cell can then increase from 20,000~50,000 to as high as 2 million[34]. HER-2 has a number of potential uses in breast cancer, including determining prognosis, predicting relative resistance to hormone therapy and adjuvant cyclophosphamide, methotrexate, 5-fluorouracil (CMF) therapy, selecting for enhanced response to adjuvant anthracycline-based therapy and identifying patients for treatment with Herceptin (trastuzumab). Most published reports on axillary node-positive breast cancer patients conclude that either HER-2 gene amplification or overexpression correlates with an adverse outcome in patients with breast cancer[35]. However, the prognostic impact of HER-2 in node-negative breast cancer patients is less clear[35]. It is important to point out that most of the studies relating HER-2 to patient outcome were retrospective in design, contained relatively low numbers of patients and used a variety of HER-2 assays. Furthermore, in many of the studies, patients received different types of adjuvant therapy, making it difficult to differentiate a prognostic from a predictive impact. Consistently with these studies, our results showed significant association between HER-2/neu immunopositivity and local-regional recurrence and metastasis.

Based on the available information, the EGTM panel recommends that HER-2 should not be used alone for determining outcome in patients with breast cancer. However, as HER-2 is being measured on an increasing number of patients with breast cancer, information on HER-2 status may be combined with standard prognostic factors for determining outcome in patients with breast cancer.

ER and PR are transcriptional factors which mediate the actions of estrogens and progesterone, respectively[36,37]. Both receptors are now known to exist in two different forms. For ER, these forms are known as ER-alpha and ER-beta[38], and for PR, the two forms are known as PRA and PRB[39]. To date, for ER, a clinical role has only been established for the alpha form. Existing assays for PR do not discriminate between the two forms. The main clinical application of ER (i.e. ER-alpha) and PR in breast cancer is selecting patients

likely to respond to hormone therapy. In both early and advanced disease, hormone receptor-positive patients have a significantly greater probability of responding to hormone therapy than patients lacking receptors[40,41]. Therefore, the EGTM panel recommends that ER (i.e. ER-alpha) and PR assays be performed on all patients with newly diagnosed breast cancer. Similar recommendations have been previously made by the EGTM[40,42], as well as by other expert panels such as the American Society of Clinical Oncology (ASCO)[40,43], the National Academy of Clinical Biochemistry, the European Society of Medical Oncology[40], the European Society of Mastology[44] and a National Institute of Health developmental panel[40]. At this stage, the assay of ER-beta cannot be recommended for predicting response to endocrine therapy. While the primary purpose for performing hormone receptor assays lies in selecting a likely response to endocrine therapy, information on receptor status or concentration may also be of prognostic value. Generally, for the first 4~5 years after diagnosis, ER-positive patients have a better outcome than ER-negative patients. However, after this period, the favorable prognostic impact of ER is lost. A further limitation of ER as a prognostic factor is that it is of little value in lymph node negative patients[40]. Although less work has been carried out on the prognostic impact of PR, patients with tumors expressing PR also tend to have a better prognosis than those lacking this receptor[41]. Consistently with other studies, in this study both local regional recurrence and metastasis were more often seen in ER negative patients and there was significant association between PR negative status and metastasis.

Since both ER and PR are relatively weak prognostic factors in breast cancer, these factors should not be used alone for differentiating between patients with indolent and aggressive breast cancers. However, hormone receptors may be combined with established prognostic factors in determining outcome.

Conclusion

P53 and HER-2/neu expression together with steroid receptors status (ER/PR status) are tissue based markers accepted in clinical practice and have an established role in predicting outcome in breast cancer patients. Thus they may be of use in selecting suitable therapeutic strategy and determining prognosis in these patients.

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