

Correlation of HER2/neu Over Expression, p53 Protein Accumulation and Steroid Receptor Status with Tumor Characteristics: An Iranian Study of Breast Cancer Patients

***M Moradi-Marjaneh¹, F Homaei-Shandiz², SAA Shamsian¹, I Eftekhari-Zadeh Mashhadi¹, MR Hedayati-Moghadam¹**

¹*Iranian Academic Center for Education, Culture & Research (A.C.E.C.R), Mashhad Branch, Iran*

²*Radiotherapy and Oncology, Mashhad University of Medical Sciences, Iran*

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Abstract

Background: Molecular biomarkers are valuable in evaluation the course of malignancies. In this study, the relationship between HER2/neu, p53, steroid receptors and clinicopathological features of patients with breast cancer has been assessed.

Methods: Three hundred thirty nine women with a primary diagnosis of breast cancer from 2001 to 2006 were included. They were evaluated for tumor features. The content of the steroid hormone receptors and expression of HER2/neu and p53 was also examined by immunohistochemistry assay.

Results: From patients arrived at this study, 52%, 36%, 46.5% and 44% were positive for HER2/neu, p53, ER and PR respectively. No association was found between most of pairs. HER2/neu expression significantly related with grade ($P=0.016$). P53 had considerable reverse relation with axillary lymph node involvement and primary distant metastasis ($P=0.010$ and $P=0.023$, respectively). A significant association was found between PR and lymph node involvement ($P=0.038$).

Conclusion: This study present the pattern of prognostic biomarkers in breast cancer for the first time in northeast of Iran and suggests to conduct larger studies regarding other effective variables include demographic ones.

Keywords: *Breast Cancer, HER2/neu, p53, Steroid Receptor, Stage, Iran*

Introduction

Several molecular markers have been detected that are important in clinical aspect of malignancies especially in breast cancer (1). Assessment of these biomarkers is valuable in predicting the outcome of disease and decision making for optimal treatment (2-5). Estrogen and progesterone receptors are found to be of great predictive and prognostic value. Previous studies have shown survival advantages among women with hormone receptor-positive tumors (1, 6, 7). HER2/neu and p53 genes are subjects of more recent investigations. HER2/neu is a member of the epidermal growth factor receptor (EGFR) family. Activation of the HER2/neu gene, located in chromosome 17q11.2-12 results in synthesis of a 185-kD transmembrane protein, whose intracellular do-

main possesses tyrosine kinase activity and through phosphorylation induction leads to tumor cell proliferation (8). HER2/neu amplification or over expression has been shown to be associated with higher tumor grade and poorer prognosis (1, 9). p53 is involved in regulating cell proliferation, including apoptosis, and in promoting chromosomal stability. It had been demonstrated that some percentage of breast cancers have mutations in tumor suppressor gene p53, which are associated with clinical aggressiveness (10, 11). Although prognostic and predictive value of these four biomarkers has been well established in previous investigations, relation between these biomarkers and histologic features of breast cancer is a point to be discussed more. Several available studies have been carried out to analyze the role of bio-

markers in patients' survival but less number have focused on the relation with tumor characteristics. In spite of common finding in such surveys, there is a significant variation in results obtained from different areas (11-16).

Breast cancer is the most common malignancy among women in Iran. Similarly, in Khorasan Province, breast cancer ranks the first among cancers of women, comprising 22.3% of all malignancies in females (17). However, researches conducted in this region mostly limited to epidemiologic studies and the lack of adequate data of biomarkers is obvious.

The present study was aimed at determination of association between mentioned molecular indicators and clinicopathological characteristics of breast cancer including tumor size, lymph node invasion, primary distant metastasis, and grade of tumor in two great educational hospitals of Mashhad which are the reference center for patients with cancer from all areas of the Khorasan Province and north-east of Iran (18, 19).

Materials and Methods

Patients

We included 339 women newly diagnosed with primary breast cancer between 2001 and 2006 lived in Khorasan Province and were treated at two great university hospitals in Mashhad, Iran (Ghaem and Omid). All patients had biopsy samples of tumor collected at the time of diagnosis that were available for immunohistochemical analysis.

Clinicopathologic determinations

The specimens were histological graded according to NCHG, a modification by Elston of the Scarff-Bloom-Richardson grading scheme (20). Clinical and pathological staging was performed using the American Joint Committee on Cancer TNM Staging System for Breast Cancer (21).

Immunohistochemical analysis

Before treatment, a specimen was obtained from each patient's tumor for evaluation of HER2/neu, p53 and steroid hormone receptors. This was performed by an immunohistochemical assay, LSAB technique. For this purpose, a 4µm sec-

tion from each submitted paraffin block were stained with H&E for histologic evaluation and verifying that an adequate specimen were presented and that quality of fixation was sufficient. Then, specific staining for each biomarker was performed. Four-micrometer-thick sections were prepared from selected blocks and float mounted on adhesive glass slides charged with polyallanine.

Tumor specimens were analyzed using the following antibodies: Polyclonal rabbit anti-human c-erbB-2 oncoprotein (DAKO, Glostrup, Denmark) at 1: 200 dilution for HER2/neu; monoclonal mouse anti human p53 protein, clone DO-7 (DAKO) at 1: 100 dilution for p53; RTU-ER-6F11 (Novocastra, Newcastle, UK) at 1: 50 dilution for estrogen receptor (ER) and RTU-PGR-312 (Novocastra) ready to use for progesterone receptor (PR).

Immunohistochemistry was performed according to manufacture instructions for assessment of HER2/neu in the cell membrane of specimen prepared. Specimens were classified as HER2/neu positive according to HercepTest™ Scoring Guidelines (Table 1) (22).

After introduction of specific antibodies, expression status of p53, ER and PR was also evaluated. The expression status of p53 was assessed regarding the proportion of nuclear staining (Table 2) (11).

Like other biomarkers, expression of ER and PR were assessed after the entire immunostained slides had been evaluated by light microscopy, and results were determined by assigning a proportion score and an intensity score according to Allred's procedure (Table 3) (23).

Statistical Analysis

Immunohistochemical results for molecular markers compared together and with clinicopathologic characteristics using the Chi-square, ANOVA and *t*-test.

Table 1: HercepTest™ Guidelines for Scoring of HER2/neu

Score to Report	HER2 Protein Overexpression Assessment	Staining Pattern
0	Negative	No staining is observed, or membrane staining is observed in <10% of the tumor cells.
1+	Negative	A faint/barely perceptible membrane staining is detected in >10% of tumor cells. The cells exhibit incomplete membrane staining.
2+	Weakly Positive (Equivocal)	A weak to moderate complete membrane staining is observed in >10% of tumor cells.
3+	Strongly Positive	A strong complete membrane staining is observed in >10% of tumor cells.

Table 2: Assessment of p53 expression

Score to Report	p53 Protein Accumulation Assessment	Staining Pattern
0	Negative	No staining is observed
1	positive	A nuclear staining is detected in <10% of tumor cells.
2	positive	A nuclear staining is detected in 10%-50% of tumor cells.
3	positive	A nuclear staining is detected in >50% of tumor cells.

Table 3: Allred scoring system for ER/PR

Proportion of tumor cells with positive nuclear staining	PS Observation	Average intensity of all positive tumor cells	IS Observation
0	None	0	None
1	> 0 to 1/100	1	Weak
2	> 1/100 to 1/10	2	Intermediate
3	> 1/10 to 1/3	3	Strong
4	> 1/3 to 2/3		
5	> 2/3 to 1		

Total Score = PS + IS

Sum of Proportion Score (PS) and Intensity Score (IS)	Interpretation
0-2	Negative
≥3	Positive

Results

In this study, 339 women with breast cancer were evaluated. The age of the patients at diagnosis ranged from 21 to 81 yr (mean 47.4±11.3 yr) and 41.7% were in post-menopausal period. Eighty-nine patients (27.2%) had positive family history for malignancy and 51.9% had a previous history of oral contraception pill (OCP) consumption. The means for age of menarche and first pregnancy were 13.3 and 20.5 yr, respectively. Malignancy was in the right breast in 49.3% of patients, and in 49.6% of cases was found in the left, while just 4 patients had bilateral tumor. Tumors were located in the upper lateral quadrants in 45.5% of cases, whereas other quadrants and locations had an involvement of lesser extent. In pathological reports, 88.2% of cancers were invasive ductal carcinoma, though other pathologic groups were also seen, but with extremely lower frequencies. Tumors were mainly in grade II (58.6%) (Table 4). T2 stage was found in 49.8% of cases, while T1 and T3 constitute lower percentage (10.5% and 28.8% respectively). In 55.2% of cases, involvement of lymph node was in N1 stage and 90.1% of patients had no distant metastasis at their first visit (M0). When evaluating axillary lymph nodes, patients had no involved in 28.6% of cases, while cases with 1-3 and more than three involved axillary lymph node constitute 33.7% and 37.7% correspondingly (Table 4). Treatment modality used for patients was mainly (94.8%) surgery plus radiotherapy plus chemotherapy. Mastectomy was performed in 94.6% of patients who had undergone surgery. Radiotherapy and chemotherapy was done in 95.8% and 99.4% of patients, respectively. From patients arrived at this study, 165 (53.4%) were estrogen receptor negative and 144 (46.6%) were ER positive (Fig. 1) (Table 5). Respective figures for progesterone receptor status were 177 (56.2%) and 138 (43.8%) (Fig. 2). 48.3% of patients were HER2/neu negative, while 165 (51.7%) were HER2/neu positive (Fig. 3). p53 receptor was negative in 63.9% of all cases, while 36.1% of patients had p53 positive results (Fig. 4).

The relations between pairs of assessed histopathological and immunohistochemical factors were analyzed, and no association was found between the majorities of pairs of factors except for four couples as described in Table 5.

A significant association was found between over expression of HER2/neu and grade of tumor. In patients who classified into grade III (poorly differentiated), 63.2% were HER2/neu positive while only 46.6% of patients with lower grades were HER2/neu positive ($\chi^2(1) = 5.8, P = 0.016$). Interestingly p53 was observed to have a significant reverse relation with lymph node involvement and primary distant metastasis. Within cases whose tumors were larger than 2 cm and patients with primary metastasis, 26% and 16.7% were positive for expression of p53, respectively. In contrast, 65% patients with smaller tumor and 40.4% patients who had not primary metastasis were p53 positive ($P = 0.010$ and $P = 0.023$, respectively). In addition, it has been shown that PR significantly associated with axillary lymph node involvement. PR positive cases consisted 43.7% of patients whose axillary lymph nodes had not been involved by tumor and just 26.5% of patients suffered from lymph node involvement ($\chi^2(1) = 4.3, P = 0.038$).

Table 4: Clinicopathological characteristics of patients with breast cancer

Factor	Value [n (%)]
n	339
Age at diagnosis (years)	
≤ 50	215 (63.4)
> 50	124 (36.5)
Age range (years)	21-81
Tumor size (cm)	
≤ 2	35 (10.8)
> 2	288 (89.1)
Number of positive lymph nodes	
0	89 (28.6)
1-3	105 (33.7)
> 3	117 (37.7)
Histological grade	
I	28 (10.5)
II	156 (58.6)
III	82 (30.8)

Table 5: Relationship between clinicopathological characteristics of breast cancer and molecular biomarkers

Factor	ER		PR		HER2/neu		p53	
	Positive/total (%)	<i>p</i>	Positive/total (%)	<i>p</i>	Positive/total (%)	<i>p</i>	Positive/total (%)	<i>p</i>
Total	144/309 (46.6)		138/315 (43.8)		165/319 (51.7)		95/263 (36.1)	
Tumor size								
T1	13/31 (42)	NS	13/31 (42)	NS	12/31 (38.7)	NS	11/27 (64.7)	NS
T2	70/149 (47)		68/153 (44.4)		77/153 (50.3)		54/126 (42.8)	
T3	37/85 (43.5)		32/85 (37.6)		47/87 (54)		20/75 (26.6)	
T4	15/31 (48.3)		16/33 (48.4)		23/34 (67.6)		6/23 (26)	
Lymph node involvement								
N0	33/79 (41.7)	NS	28/81 (34.5)	0.038	38/80 (47.5)	NS	26/65 (40)	0.010
N1	69/154 (44.8)		68/160 (42.5)		86/162 (53)		49/131 (37.4)	
N2	19/41 (46.3)		22/40 (55)		23/43 (53.4)		9/33 (27.2)	
N3	5/5 (100)		4/5 (80)		1/5 (20)		2/4 (50)	
Primary distant metastasis								
M0	110/247 (44.5)	NS	107/253 (42.2)	NS	131/257 (51)	NS	86/213 (40.3)	0.023
M1	12/29 (41.3)		15/29 (51.7)		18/30 (60)		4/24 (16.6)	
Histological grade								
I	16/27 (59.2)	NS	17/26 (65.3)	NS	12/26 (46)	0.016	11/22 (50)	NS
II	70/139 (50.3)		68/145 (46.8)		69/148 (46.6)		49/128 (38.2)	
III	31/78 (35.6)		31/81 (38.2)		49/79 (62)		21/63 (33.3)	

NS: not significant

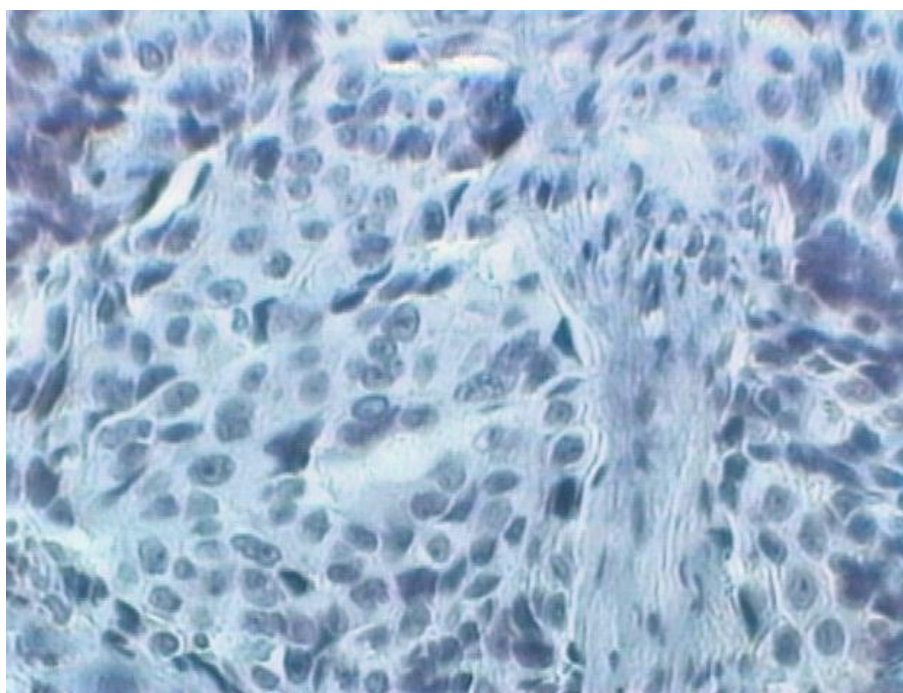


Fig. 1A: Breast carcinoma shows no nuclear staining for ER (20x magnification)

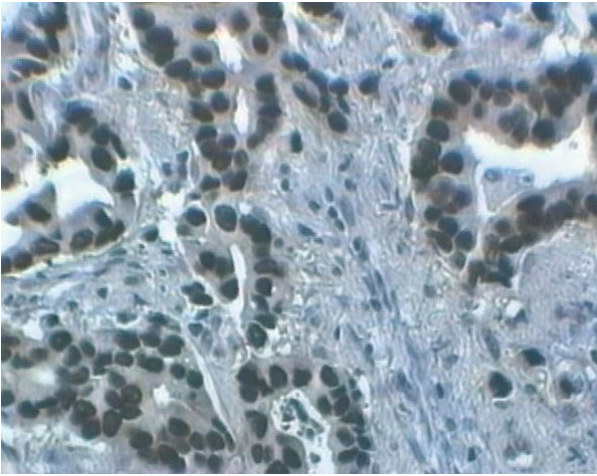


Fig. 1B: Breast carcinoma shows strong nuclear staining for ER (20x magnification)

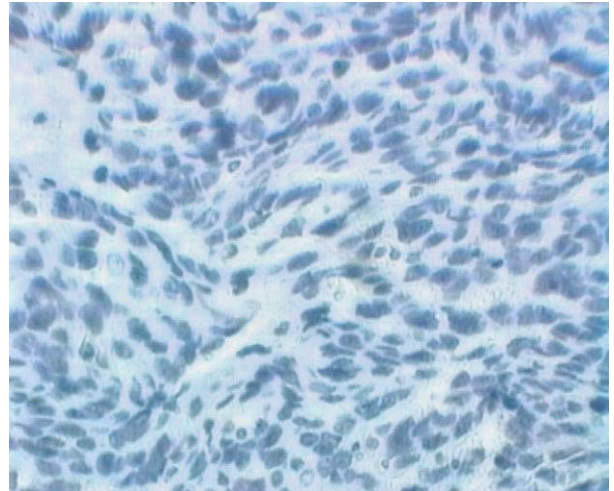


Fig. 3A: Breast carcinoma with no membranous staining for HER2/neu protein. Score 0 (20x magnification)



Fig. 2A: Breast carcinoma shows no nuclear staining for PR (20x magnification)

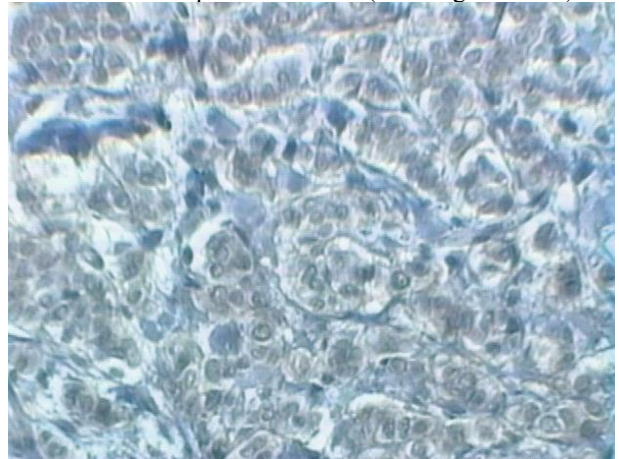


Fig. 3B: Breast carcinoma with faint incomplete membranous staining for HER2/neu protein. Score 1 (20x magnification)

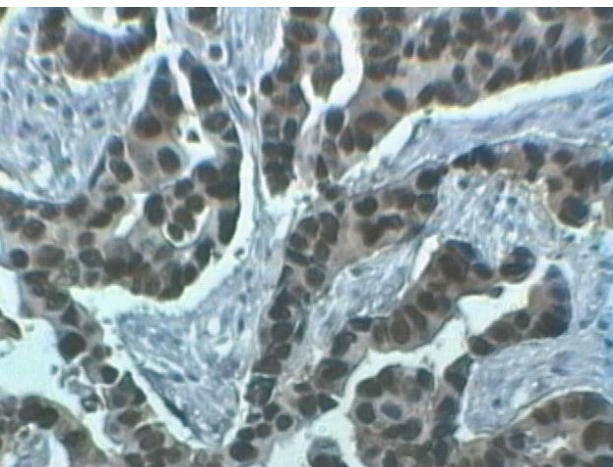


Fig. 2B: Breast carcinoma shows strong nuclear staining for PR (20x magnification)

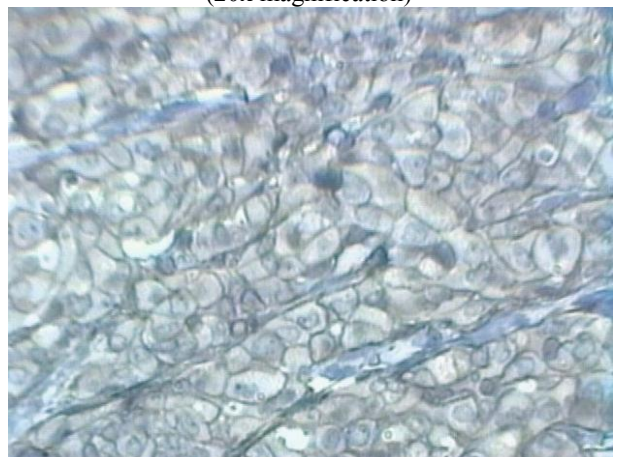


Fig. 3C: Breast carcinoma with moderate complete membranous staining for HER2/neu protein. Score 2 (20x magnification)

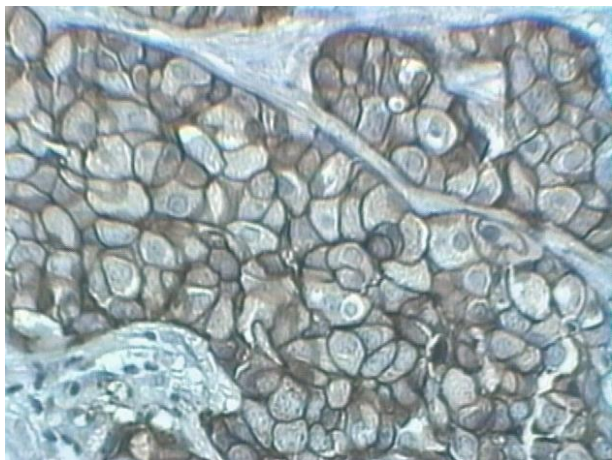


Fig. 3D: Breast carcinoma with strong complete membranous staining for HER2/neu protein. Score 3 (20x magnification)

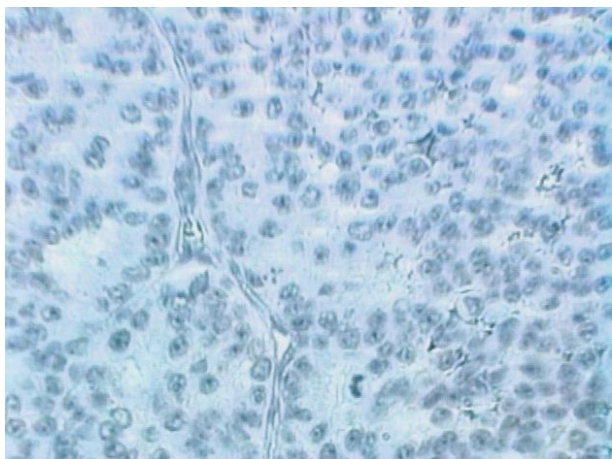


Fig. 4A: Breast carcinoma shows no nuclear staining for p53 protein (20x magnification)

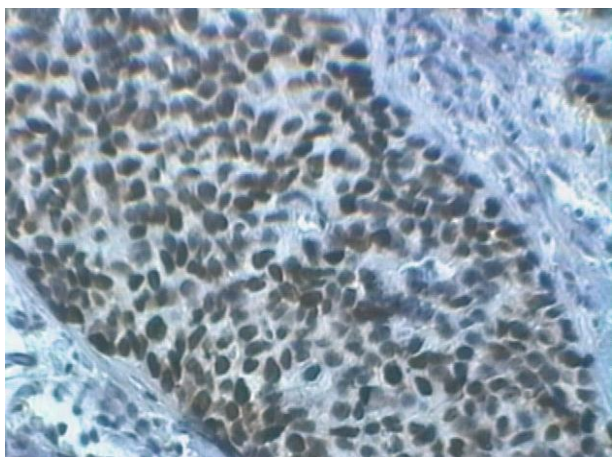


Fig. 4B: Breast carcinoma shows strong nuclear staining for p53 protein (20x magnification)

Discussion

Determination of factors affecting clinicopathological features of breast cancer is important in improving insight toward this disease. Some studies have evaluated prognostic factors in Iranian women suffered from breast cancer (24-26). Our study expands on such studies, more evaluating the relationship between hormone receptors, HER2/neu and p53 status and tumor characteristics (including grade, size, axillary lymph node involvement and primary distant metastasis).

Women with hormone receptor-positive tumors were frequently shown to have survival merits in the comparison with women with hormone receptor-negative tumors. Additionally, hormone receptor positivity usually is accompanied by lesser grade and stage (11, 27). Nevertheless, our study shows the different pattern of finding. Despite hormone receptor contents had no noticeable relation with grade, tumor size and primary distant metastasis, PR significantly associated with lymph node involvement. Therefore, although it is documented that these receptors are important prognostic and predictive factors of breast cancer (1-3), their role in stage and grade of Iranian women's tumors is suspected by this study.

According to previous studies, ER and PR are indicators of lower risk of mortality after diagnosis (1-3). Immunohistochemistry in formalin-fixed paraffin-embedded tissue is the newest technique for ER and PR Assays. The most common and important problems associated with IHC is the lack of standardization. The majority of laboratories around the world utilize different reagents with unequal sensitivities and specificities; various detection systems; and diverse scoring methods. Additionally, most laboratories use arbitrary definitions of positive or negative. Consequently ER assays are estimated to be inaccurate approximately 20% of the time. This figure may be even higher for PR, since laboratories have less experience assaying PR by IHC (28). The present study is not excluded. Thus the revealed difference between findings of this study and previous ones maybe partly because

of this problem. In addition, some other area-specific factors include inhabitants' race and ethnicity may affect biomarkers presentation and rationalize these results. Previous findings have shown that breast cancer is a heterogenous disease composed of various biological subtypes across racial lines (29-31). Furthermore, the relationship between molecular factors and clinicopathological features reported from different countries are not well-matched (24, 32-35). There is no similar conducted research in this region of the country. By lack of analogous studies in this area, this study can suggest a new pattern of hormone receptors content in north east of Iran. HER2/neu over-expression and p53 protein accumulation participate in pathogenesis of breast cancer. They are indicators of poor prognosis in breast cancer and usually correlated directly with histological grade and stage components of breast cancer (1-3, 11). According to data of this study, no relation was found between HER2/neu and stage features mentioned above. But it was significantly associated with higher grade which is compatible to prior studies (1, 11). Conversely, p53 had the evident reverse relation with lymph node involvement and primary distant metastasis. Mentioned possibilities for hormone receptors include the assay used, the scoring system applied and the characteristics of the patient population could justify this pattern to some extent.

Observing these variations in the association between molecular biomarkers and tumor characteristics perhaps refer to limitations of this study, which had limited number of cases with different kind of breast tumor, and in different age groups. Although nearly 90% of them had invasive ductal carcinoma, they had variety longevity at date of diagnosis. It has been shown that some usual prognostic factors are age dependent, resulting in a worse prognosis in younger patients (36).

In conclusion, this study present the pattern of prognostic biomarkers in breast cancer for the first time in northeast of Iran which is not compatible to results of previous studies. However, to generalize these findings, it is necessary to

carry out larger studies regarding other effective variables include demographic ones.

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