Evaluation of the relationship between human epidermal growth factor receptor–2/neu (c–erbB–2) amplification and pathologic grading in patients with breast cancer

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ABSTRACT

Objectives: The human epidermal growth factor receptor-2 (HER–2)/neu is a proto-oncogene that is amplified in 10-30% of breast cancers. It is known to be associated with a poor overall survival. We studied the relationship between its amplification and different histological gradings of breast cancer.

Methods: We studied 196 patients diagnosed with breast cancer in 2005 at the Omid and Ghaem Training Hospital, Mashhad Medical University, Iran. The HER–2/neu oncoprotein was measured by immunohistochemistry and the histological gradings were carried out according to the Bloom-Richardson Grading system.

Results: Sixty-seven (34.2%) cases were HER-2/neu positive and 129 (65.8%) cases were HER-2/neu negative. Overexpression of HER-2/neu was significantly higher in breast cancer patients <30 years (50% versus 33.3%, p=0.034). There was a non-significant statistical relationship between histological grading and overexpression of HER-2/neu oncogen (p=0.087). Twelve (17.5%) of HER-2/neu positive cases were metastatic and only 4 (3.1%) of HER-2/neu negative cases had metastasis (p=0.051).

Conclusion: HER-2/neu gene amplification or its overexpression is detected in approximately 34.2% of breast cancer cases. Patients with HER-2/neu positive breast cancer have higher stage and grade diseases. This may help to use a better treatment for patients.

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Overexpression of the human epidermal growth factor receptor-2 (HER–2)/neu (also known as c-erbB-2, oncoprotein is positive in approximately 25-30% of breast cancer cases. It is associated with poor overall survival as it enhances the metastatic potential of breast cancerous cells and induces resistance to drugs and tumor necrosis factor, which allows malignant cells to escape from the host's immune system.[1-6] Human epidermal growth factor receptor-2/neu positivity was found to be proportionately associated with higher grades in infiltrating ductal carcinoma.[7] The HER-2 /neu gene encodes a 185–kDa transmembrane receptor tyrosine sinkinase with homology to members of the epidermal growth factor receptor family. Human epidermal growth factor receptor-2/neu blocks tumor necrosis

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factor–induced apoptosis and reduces the host's defenses against cancer. Human epidermal growth factor receptor-2/neu overexpression in breast cancer cells serves as an excellent target and a prognostic guide for the development of anti-cancer drugs, such as (Trastuzumab-HER-2/neu antibody), which is used for treatment of breast cancer.[8] The aim of this study was to investigate the relation between HER–2/neu oncoprotein and histopathological characteristics (mainly gradings) in women with breast cancer.

Methods. One hundred ninety-six patients with breast cancer all diagnosed in 2005 from Omid and Ghaem Hospitals of Mashad Medical University in Iran were selected. Histological sections of cancerous tissues and hematoxylin-eosin (H&E) slides were reviewed by our pathologist and were graded according to the Bloom-Richardson grading system.[9] The paraffin blocks of cases were cut into 4 mm-thick slices and dyed with immunohistochemical stains by means of labeled strepto-avidin-biotin method. Standard Hercept test procedure (Dako 5204) was used to stain the prepared samples.[7] The immunohistochemical staining of Hercept test was scored by comparing them with control slides. The positivity of HER–2/neu oncoprotein was evaluated according to the scoring system Dako Hercep Test. In this system, score of zero represents the condition in which either the membrane is not stained, or it is stained in <10% of tumor cells. In this score, HER-2/neu overexpression assessment was negative. In score (1+), a faint membrane staining was detected in >10% of the tumor cells and HER-2/neu overexpression assessment was negative. A weak to moderate staining of the entire membrane was observed in >10% of the tumor cells in score of 2+ which was weakly positive. Finally, a strong staining of the entire membrane was observed in >10% of the tumor cells in score of 3+, which is strongly positive. In our study, we considered tumors with IHC 0, 1+, 2+ as Her-2/neu negative, and 3+ cases as Her-2/neu positive.[7] Histological grading of breast cancer is based on Bloom-Richardson Grading system. The grading score was measured according to the tubule formation, nuclear pleomorphism and mitotic count.[9]

Statistical analysis. Data were analyzed with SPSS soft-ware (version 11.5) and a $p \leq 0.05$ was considered significant. Chi-square and t-tests were adopted for comparing qualitative and quantitative variables, respectively.

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Results. The characteristics and demographics of breast cancer patients are shown in **Table 1**. There were 173 patients with infiltrating ductal carcinoma and 13 with invasive lobular carcinoma and the rest suffered from other pathologies. One hundred thirteen (57.6%) patients were <50 years of age (ranging from 21-81) and 10 (5.1%) of them were <30. One hundred twenty-three (62.8%) patients were premenopausal. Staging was compared with AJCC staging system 2002 and the most frequent stages were II and III (67.43%). The mean metastatic axillary lymph nodes were 4.05 nodes (ranging from 0 to 24). Overexpression of HER-2/neu oncogen was evaluated in 196 patients. One hundred twenty-nine cases (65.8%) were HER-2/neu negative and 67 (34.2%) bore HER2-/neu overexpression. As with the age of the patients 37 (55.2%) out of 67 patients aged <50 and 30 patients (44.7%) >50 had an overexpressed HER-2/neu (p=0.061). In young women (aged <30), 5 (50%) had overexpressed HER-2/neu but 62 (33.3%) (>30 years) patients with breast cancer were positive for HER-2/neu (p=0.034). Overexpression of HER-2/neu oncogen was significantly higher in younger females (<30 years). The relationship between age and HER-2/neu status was shown in **Table 2**. Grading was carried out on the basis of Bloom- Richardson

Table 1 - Distribution of clinico–pathological characteristics in patients with breat cancer (n=196).

Characteristics	n	(%)
Age (years)		
Mean	46.21	
Range	21-81	
Size of tumor (cm)		
Mean	4.15	
Range	0.5-14	
T1	27	(14.0)
T2	96	(49.7)
T3	54	(28.0)
T4	16	(8.3)
Unknown	3	
Number of axillary dissection nodes		
Mean	8.11	
Range	0-24	
Number of positive nodes		
Mean	4.05	
Range	0.24	
N0	51	(29.1)
N1	100	(57.1)
N2	22	(12.6)
N3	2	(1.1)

Table 2 - Correlation between age and Her-2 status

Age	Her-2 negative (n=129)	Her-2 positive (n=67)	*P* value
<50	76 (67.2)	37 (32.7)	
≥50	53 (63.8)	30 (36.1)	0.061
<30	5 (50)	5 (50)	
≥30	124 (66.6)	62 (33.3)	0.034

system according to the tubule formation, nuclear pleomorphism and mitotic count. Nine (7.1%) of HER-2/neu negative patients were grade I, 77 (59.6%) grade II and 43 (33.33%) grade III. Forty-five (67.5%) of patients positive for HER-2/neu were grade II, while 20 (29.7%) were grade III. Only 2 (2.8%) of the HER-2/neu positive patients were grade I (p=0.087). About the relationship between the tumor size and HER-2/neu status, we found that 35 (52.2%) of HER-2/neu positive cases have larger than 5 cm tumors and 51 (39.5%) of HER-2/neu negative patients have the same size tumors (p=0.107). Forty-one (61.2%) of patients with HER-2/neu positive had ≥4 positive axillary lymph nodes but 71 (55%) of HER-2/neu negative patients had ≥4 positive axillary lymph nodes (p=0.538). As far as the tumor stage is concerned, 12 (17.5%) of HER-2/neu positive patients were metastatic and only 4 (3.1%) of HER-2/neu negative patients had metastasis (p=0.051) (**Table 3**).

Table 3 - Clinical and histopathological characteristics in Her-2 negative and positive patients.

Characteristics	Her-2 negative		Her-2 positive		*P* value*
	n	(%)	n	(%)	
Total	129	(65.8)	67	(34.2)	
Age					
<50	76	(67.2)	37	(32.7)	0.061
≥50	53	(63.8)	30	(36.1)	
Stage					
I	38	(29.45)	15	(22.5)	0.051
II	49	(37.98)	25	(37.5)	
III	38	(29.45)	15	(22.5)	
IV	4	(3.1)	12	(17.5)	
Size of breast mass					
<5 cm	78	(60.5)	32	(47.8)	0.107
≥5 cm	51	(39.5)	35	(52.2)	
Number of positive node					
0	32	(24.8)	12	(17.9)	0.538
1-4	26	(20.2)	14	(20.9)	
≥4	71	(55)	41	(61.2)	
Grading					
I	9	(7.1)	2	(2.8)	0.087
II	77	(59.6)	45	(67.5)	
III	43	(33.33)	20	(29.7)	

*p values from chi-square or fisher's exact test (p>0.05 = significant)

Discussion. Breast carcinoma is a heterogeneous disease in terms of its clinical behavior. Clinical and pathological variables such as tumor size, histologic grading, histologic type, lymph node metastases, vascular space invasion, tumor cell proliferation, tumor necrosis, extent of ductal carcinoma in situ, age, and pregnancy may help predict prognosis and the need for adjuvant therapy.[9] Newer prognostic factors and predictors of the tumor's response to a treatment are needed, to distinguish subgroups with different biological features. Otherwise, they appear homogeneous if judged solely upon classic pathological and clinical criteria. Estrogen and progesterone receptors (ER-PR) and Her-2/neu make the most acceptable factors for predicting prognosis, response, resistance to treatment, and potential use of newer drugs such as trastuzumab in the case of Her-2/neu overexpression. In this study, we found that 67 (34.2%) of 196 cases were Her-2/neu positive. Although there is a wide variation in Her-2 overexpression and amplification, our figure appears to be slightly higher than the commonly accepted rate of 20-30%.[10-14] It does appear, however, to be lower than those reported in Eastern Asia[15,16] and in countries such as Lebanon[17] and Egypt.[18] We found a negative correlation between Her-2/neu overexpression and age in this study. Patients <30 years of age were more likely to have overexpressed Her-2/neu than patients of >30 years old (50% versus 33.3%, p=0.034).It should be pointed out that the higher rate of Her-2/neu overexpression in young patients has been documented in a number of previous studies.[18-20] Our results show that the probability of having an overexpressed Her-2/neu increases as the tumor gets larger. Tumor fractions larger than 5 cm tended to have higher rates of Her-2/neu expression than those of 2 to 5 cm size (52.2% versus 39.5%), but this difference was not statistically significant (p=0.107). Other groups have shown a direct relationship between lymph node metastases and Her-2/neu expression.[21-23] Our data revealed that 41 (61.2%) of Her-2/neu overexpressing tumors had ≥4 lymph node metastases, whereas only 71 (55%) of Her-2/neu negative cases were so. However, this difference was not statistically significant (p=0.538).

We believe that the low number of cases with known nodal status is responsible for the lack of significant correlation in our study; therefore, future studies with larger numbers of patients are needed to confirm the association of Her-2/neu expression with nodal metastases. Similarly, we were unable to show a significant relationship between Her-2/neu expression and the histologic grading of breast carcinoma. Other studies concluded that Her-2/neu expression or amplification is associated with the grading system.[19,21,23,24] It should be pointed out, however, that the low number of cases in grade I carcinomas (11 cases) in our study would not allow us to evaluate this variable with any degree of confidence. According to our study a strong relation between Her-2/neu overexpression and associated metastatic disease at time of presentation was proved (17.5% versus 3.1%, p=0.051). This conclusion was reached because of the reports indicating the concordance between the HER-2/neu status of the primary tumor and metastases in patients.[13,14] Several papers strongly support these facts in patients with breast carcinoma.[15-17] In pathology laboratories, the evaluation of HER–2/neu status begins with an immunohistochemical analysis for the expression of HER-2/neu protein. This method that we also used is simply compared to the other procedures such as fluorescence in situ hybridization (FISH) but the latter method (FISH) is more accurate and more predictive of a favorable response.[12] These researches developed the concordance of high cell proliferation index (Ki-67), HER–2/neu amplification and negative hormonal receptor presence and a high risk of recurrence in patients with breast cancers.[17-21]

In conclusion, as far as the age is concerned, 32.7% of the females <50 years old and 36.1% >50 years old had overexpressed HER-2/neu (p= 0.061). Fifty percent of the patients <30 overexpressed HER-2/neu and 33.3% of female breast cancers in >30-years old were HER-2/neu positive (p=0.034). Overexpression of HER-2/neu is much more seen in younger age groups. Only 2 of the HER-2 positive patients was grade I. 61.2% of patients with HER-2/neu positive had 4 or more positive axillary lymph nodes. 17.5% of the HER-2/neu positive were metastatic but 4 cases of the HER-2/neu negative had metastasis. In this study, we evaluated the relationship between HER 2/neu status and the pathologic grading in 196 breast cancer cases by immunohistochemical technique. 67 cases (34.2%) were HER-2 /neu positive. which is similar to the other studies. as far as the age is concerned, had metastasis. Finally, patients with HER-2/neu positive breast cancer have higher stage and probably higher grade disease. This may help to use a better treatment for patients.

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