Correlation of HER-2 Status with Estrogen, Progesterone Receptors and Histological Grading in 122 Invasive Breast Carcinomas

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ABSTRACT

Background and Objectives: Many laboratory & clinical studies have suggested that there are important inverse relationships between expression of HER-2 oncoprotein and hormonal receptors(ER,PR),the prognosis & treatment are also greatly depend on hormonal receptor of neoplastic cells to define these relationships using standard immunohistochemistry for HER-2,ER and PR studies were performed in addition to routine histopahtological grading of carcinoma of the breast.

Methods: Between January 2006 and December 2008 at histopathology lab of Rezgary hospital & private lab, (122) women who were operated on for diagnostic excisional biopsies of the breast cancers to allow analysis of routinely resected tissue. Quantitative expression of HER-2,ER and PR was measured on the resected samples of the breast cancers with cutoff points HER-2,ER and PR positivity were 10.6%,5.5%,5% respectively in addition to routine study of histopathology for grading of cancers according to Nottingham modified Bloom & Richardson criteria.

Results: Immunohistochemical analysis for ER, PR, and HER-2 were performed. ER and PR expression were decreased significantly in HER-2 positive tumors compared with HER-2 negative tumors (ER,15.57%,vs 41.8% vs. and PR, 13.11% vs. 37.70%;). Even among HER-2+ tumors, the rate of ER or PR expression in high-grade tumors was significantly decreased compared with intermediate-grade tumors. HER-2 was positive in 73.3% of grade 2 and 66.6% of grade 3 ductal carcinomas and 6.89 in grade 1 ductal carcinomas. HER-2 expression essentially in grades 2 and 3 ductal carcinomas, very few cases in grade 1 and correlated inversely with ER or PR expression. Although ER or PR expression is decreased in HER-2+ tumors, a substantial proportion of them still express ER or PR.

Conclusions: The results of this study are consistent with the indication of inverse relationships between HER-2 and ER,PR increased ER and PR levels in low grade neoplastic tissue ,the low grade neoplasms responding to hormonal therapy while high grade responding to hormonal therapy while high grage responding to herceptine .

Key words: HER-2, ER, PR, breast cancers .

INTRODUCTION:

Breast cancer is the most common carcinoma in women and accounts for 43.9% of all female cancers in our locality during last three years, which is more frequent than the prevalence of cancer in women at other parts of the world.[1] Prognosis and management of breast cancer are influenced by the classic variables such as histological type grading, node status, status of hormonal receptors– estrogen receptor (ER) and progesterone receptor (PR)–of the tumor, and, more recently, HER-2 status.[2,3] HER-2/ *neu,* also known as c- *erb* B-2 (*HER-2*), a proto-oncogene located on chromosome 17, is amplified and/or the protein (HER-2) over expressed in 15% to 25% of invasive breast carcinomas and is associated with a worse clinical outcome.[3,4] In contrast, ER is expressed in 70% to 95% of invasive

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lobular carcinomas and in 70% to 80% of invasive ductal carcinomas, and PR is expressed in 60% to 70% of invasive breast carcinomas[5,6]. Expression of ER and/or PR generally is associated with a better outcome. Survival and response to hormone therapy are most favorable among women with tumors positive for both ER and PR, intermediate for tumors discordant on receptor status and least favorable for tumors negative for both][7, 8]. The interrelationship of ER, PR, and HER-2 has come to have an important role in the management of breast cancer. It has been shown that patients with breast carcinoma over expressing HER-2 do not respond to tamoxifen therapy[9]. Although HER-2 expression generally is inversely correlated with ER and PR expression,[10-12] the precise extent of its inverse relationship and its association with classic histological prognostic indicators has not been studied systematically in a large series of cases. in the present study, the standardized immunohistochemically based HercepTest (DAKO)–approved methods for HER-2 testing, were used to examine the correlation between HER-2 status and expression of ER and PR and other histological features in 122 cases of consecutive and selected invasive breast carcinoma tested at our Laboratories.

MATERIALS AND METHODS:

During a 3-year period, most invasive breast carcinomas, received in our department were included in the study. ER, PR, HER-2 immunohistochemical, analyses were performed successfully in a total of 122 breast cancers as part of the routine diagnostic workup. Histological grading features of each tumor were obtained. All tests were performed in the Department of Pathology, Rezgary hospital & private lab of pathology.

Histological Examination: Histological assessment of tumor type and grade were performed routinely on 4- to 5-µm-thick H&E-stained sections of formalin-fixed, criteria outlined in the World Health Organization Classification of Tumors.[13] Briefly, the nuclear grades of primary carcinomas were designated as follows: 1. small, regular uniform cells, 2. moderate increase in size and variability: 3. marked variation in size and shape. The architectural grades of ductal carcinomas were designated as follows: 1. well-differentiated (>75%) tubule formation; 2. moderate (10%-75%) tubule formation; 3. ittle or no (<10%) tubule formation. Mitotic counts were also assessed in most of the tumors accordingly grade 1 numbers of mitosis (up to 10/10HPF) grade 2(up to 11-20/10HPF) grade 3 more than (20/10HPF[)] [1 3] .

Immunohistochemical Analysis: Tissue sections (4-5 µm thick) were used for all immunohistochemical analyses. The CONFIRM anti-ER (code number N 1575) (clone ID5) and anti-PR (code number N 1630) (clone PgR 636) monoclonal antibodies (Dako) were used for immunohistochemical analyses of ER and PR, respectively, performed by manual procedure according to the manufacturer's instructions. The ER and PR results were screened manually and interpreted as positive when more than 10% of tumor cells showed positive nuclear staining. HER-2 immunohistochemical analysis was performed using the HercepTest kit (code number Mr. 0485 polyclonal antibody) (clone PN2A) according to the manufacturer's instructions, and results were interpreted manually as follows: 0, no membrane staining; 1+, faint, partial membrane staining; 2+, weak complete membrane staining in more than 10% of invasive cancer cells; 3+, intense complete membrane staining in more than 10% of invasive cancer cells. (Dako catalog 2008).

Statistical Analyses: Statistical parameters as mean, standard deviation & chi-square test were used in the study.P value<0.05 was regarded as significant, P.values<0.01 were regarded as highly significant.

RESULTS:

The results of HER-2 immunohistochemical analyses on all tumors are summarized in (Table 1).

Table (1): Correlation of Immunohistochemical Analysis for HER-2 .

Imunohistochemical score	HER-2(Number of cases)	%
0	37	30.3
+1	14	11.5
+2	53	43.4
+3	18	14.8

P value =0.001

HER-2 was expresse (immunohistochemical score of 2+ or 3+) in 58.2% of tumors (71/122) and (score 1+) in 11.5% of tumors (14/122). While HER-2 negative tumors were 30.3 %(37/122).

The expression of ER or PR was decreased significantly in HER-2 positive tumors (score 2+and 3+) in comparison with HER-2 negative tumors (Table 2)

Table (2): HER-2 Status and ER and PR Expression.

Result	HER-2	ER	PR	ER+PR
Negative	37(30.32%)	52(42.62%)	60(49.18%)	64(52.45%)
Positive	85(69.68%)	70(57.38%)	62(50.82%)	58(47.55%)
Total	122	122	122	122

P.value=0.0013

Table (3): association between HER-2 status and (ER.PR) expression.

HER-2 , numbers	ER positive numbers	PR positive	ER+PR positive
85(69.67%) positive	19(15.57%)	16(13.11%)	8(6.55%)
37(30.3%) negative	51(41.8%)	46(37.70%)	42(34.42%)
Total	70	62	50

P value< 0.01

However, a substantial number of HER-2+ (score 1+) tumors still expressed ER or PR which included with HER-2 negative tumors in (Table 3) only.

When HER-2 status was analyzed according to the histological grading of tumors, we found that HER-2 positivity virtually was more prominent in grade 2(27%) and 3 (26%) invasive ductal carcinoma, while ER more positive in grade 2(31.14%) and grade 1(23.77%) only (2.45%) in grade 3. PR more positive in grade 2(27%) and grade 1(23.77%) while totally absent inn grade 3(zero %) (Table 4).

Table (4): HER-2, ER, and PR Status in Different grades of breast carcinoma types.

Grade of neoplasm	Her-2	ER	PR	Total
G1	2(1.63%)	29(23.77%)	29(23.77%)	29
G2	33(27%)	38(31.14%)	33(27%)	45
G3	32(26.22%)	3(2.45%)	0	48

P value =0.001

Regarding age factor and carcinoma of the breast; the majority of patients were belong to age group(36-45) 50% followed by age group (above 46 years)27.86% then age group (25-35 years) 22.13%. Most of score 3 positive HER-2 are belong to age groups more than 36 years (94.5%) usually the majority of young age groups either negative(Figure1) or score 1(Figure 2) and 2(Figure 3) only 5.5% are showing score 3 (Figure 4) positive for HER-2 table,5 and table 6.

Table (5): Correlation between scoring of HER-2 & age groups

Imunohistochemical score HER-2	25-35	36-45	46 & above
0	6(4.91%)	21(17.21%)	10(8.2%)
+1	1(0.81%)	8(6.55%)	5(4.1%)
+2	19(15.57%)	16(13.11%)	18(14.75%)
+3	1(0.81%)	16(13.11%)	1(0.81%)
Total	27(22.13%)	61(50%)	34(27.86%)

P.Value =0.001

Table (6): Correlation between score 3 positive HER-2 and age groups.

HER-2 positive score 3	Age <36	Age>36	Total
Number of cases	1	17	18
% of cases	5.5	94.5	100%

P. Value=0.002

As shown in Table 4, HER-2 positively was associated more strongly with higher histological grade in primary carcinomas. Only 2(1.63) of the grade 1 primary (including tubular carcinomas) was HER-2 positive. The majority of ER (Figure 5) and PR positive are score 3(85.25%), (54.85) respectively. Another notable expected finding, as shown in Table 4, was that the vast majority of HER-2+ tumors were grade 2 and 3 tumors. Furthermore, the rate of PR expression in nuclear grade 2 HER-2+ tumors was significantly higher than that in nuclear grade 3 tumors (ER and PR, $P < .0001$), demonstrating the inverse correlation of tumor nuclear grades and ER and PR expression even among HER-2+ tumors (Table 4). This difference also was seen when HER-2+ tumors were stratified based on architectural grade, although not as dramatically as in nuclear grade (Table 4).

Table (7): Scoring of ER and PR positives.

Scoring types	ER positive	PR positive
1+	Zero	17(27.41%)
2+	9(14.75%)	11(17.74%)
3+	61(85.25%)	34(54.85%)
Total	70	62

P.Value =0.0014

Score1= (6-20%) Score 2= (21-49%) score 3= (50% and above)

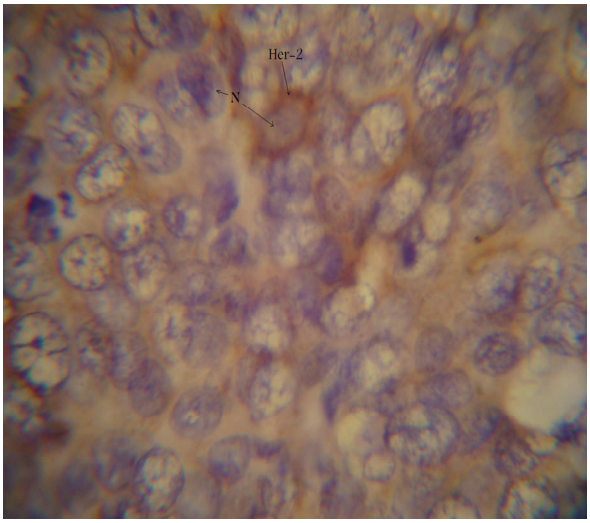

Figure (1): Immunostaining is negative (HER-2, ×1000).

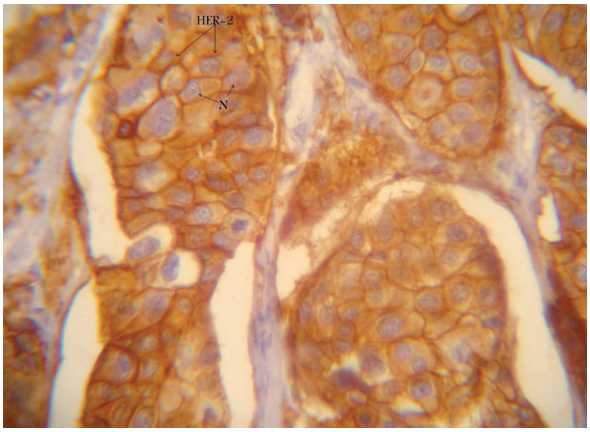

Figure (2): HER-2 score1=+I:mmunohisto–chemical staining for HER-2 X1000

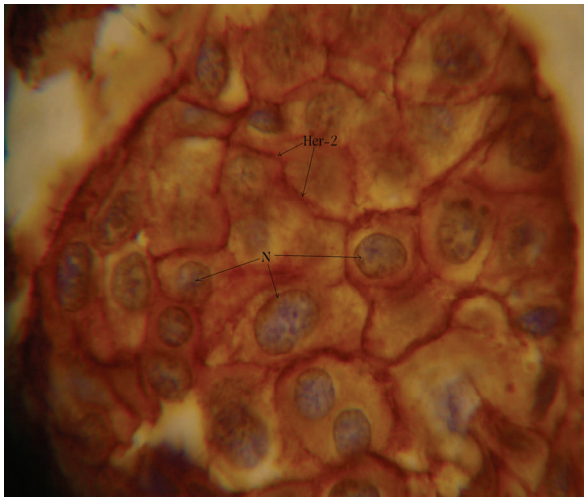

Figure (3): HER-2 score 2=++: Immunohi-stochemical staining fro HER-2X1000.

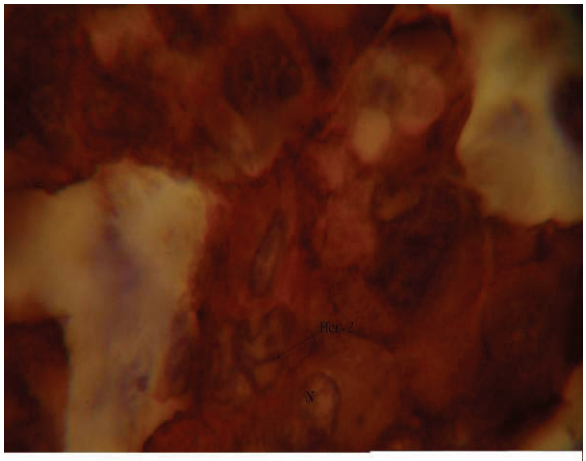

Figure (4) : HER-2 positive score 3=+++ Immunohistochemical staining X1000

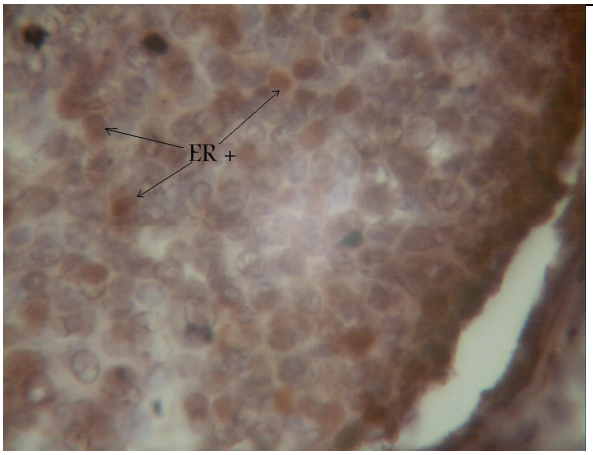

Figure (5) :ER ++ Immmohistochemical staining for ER X1000,the picture of PR + also exactly similar to this figure.both of them are nuclear receptors .

DISCUSSION:

grade in other parts of the world. However, only this study has examined these relationships in selected breast carcinomas in this region. We performed this retro and prospective study to correlate HER-2 status with other routinely used tumor characteristics such as grade of neoplasm and hormonal status in primary breast carcinomas. Which is widely adapted now days in clinical practice .Our data were showing a significant numbers of breast cancers were positive for HER-2(85%), ER (57.38%) and PR (50.82%) which is inversely related and consistent with those of other published studies in that ER and/or PR expression in general is correlated inversely with HER-2 expression? However, a substantial number of HER-2+ tumors still expressed ER (15.57%) or PR (13.11%). In most clinical practice, tumors with immunohistochemical scores of 0 or 1+ generally are considered HER-2–[14]. They constituted approximately 11.47% of all breast carcinomas in the present study. Biologically, these tumors indeed might behave more similarly to HER-2– tumors, because such patients didn't respond to trastuzumab-based therapy [15]. The correlation between HER-2 expression and tumor grade in this study were 1.63%, which were differed from other studies carried out in a large series of cases by Thor Ann et al, [16] who found HER-2 expression rates of 3.9%, 20.4%, and 38.9% in tumors of grades 1, 2, and 3, respectively. Bánkfalvi et al [17] found HER-2 expression in 4.1% of grade 1 invasive ductal carcinomas, 16.3% of grade 2 ductal carcinomas, and 30% of grade 3 ductal carcinomas, and also Hoff et al [18] in a study of 388 cases found that HER-2 was expressed in fewer than 1% of grade 1, 17% of grade 2, and 23% of grade 3 tumors. Over all studies indicated that minority of grade 1 were positive for HER-2 but majority of HER-2+ tumors were grade 2 and 3, with rates of ER and PR expression in grade 2 tumors were significantly higher than those of grade 3 tumors, even when they were HER-2+. These findings suggest that ductal carcinomas of intermediate grade also exhibit intermediate features of HER-2, ER and PR expression .

RECOMMENDATION :

1. For all breast cancers study of HER-2, ER and PR should be performed before starting any kind of treatment .
2. Study of oncogen erb.B (HER-2) over expression and gene amplification is important to evaluate prognosis and management of the patients .

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