

Original paper

Correlation and frequency of HER-2/neu Status With Estrogen and Progesterone Receptors in Breast Carcinomas

Nazar J. Metib^{^*}, Haider Jebur Kehiosh[^], Sabah K. Hamzah[^]

[^]Al-Hussein medical city, Karbala, Iraq.

Abstract

Background: Breast cancer is the most common malignant tumor in Iraqi females accounting for a third of registered female cancers. Prognosis and management are influenced by the classic variables such as histologic type and grade, in addition to tumor size, lymph node status, status of hormone receptors of the tumor and HER-2/neu status. 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) overexpressed in 15% to 25% of invasive breast carcinomas and is associated with a worse clinical outcome. The interrelationship of ER (estrogen receptor), PR (progesterone receptor), and HER-2 expression in invasive breast cancer has come to have an important role in the management of breast cancer. It has been shown that patients with breast carcinoma overexpressing HER-2 do not respond to tamoxifen therapy.

Objectives: To evaluate HER-2/neu overexpression in breast cancer by immunohistochemical study and to study the correlation between HER-2/neu status and expression of ER and PR receptors and other clinicopathological features in invasive breast carcinoma.

Patients and methods: Immunohistochemical analyses were performed successfully for ER, PR, HER-2/neu by streptavidin biotin indirect immunohistochemical methods using monoclonal antibodies for 41 specimens of formalin-fixed, paraffin embedded breast cancer tissue.

Results: There was a significant difference in HER-2/neu overexpression between invasive ductal carcinoma of NOS (Not Otherwise Specified) type from other histopathologic types (lobular, and specified type ductal carcinoma). No significant correlation was observed between HER-2/neu overexpression and higher histological grades of invasive ductal carcinoma. The expression of ER or PR was decreased significantly in HER-2/neu positive tumors in comparison with HER-2 negative tumors. Only 7.3 % of all cases (3/41) were negative for estrogen, progesterone receptors (ER/PR) and HER2/neu oncoprotein (Triple negative breast cancers). The two cases of lobular carcinoma and the three cases of the special-type carcinomas were negative for HER-2/neu.

Conclusions: HER-2/neu protein was expressed more in ductal carcinoma (NOS type) than in lobular or in carcinoma of special type and frequently detected in higher-grade invasive ductal carcinoma tumors. The expression of ER or PR was decreased significantly in HER-2/neu positive tumors. HER-2/neu positivity was not correlated with other clinicopathological parameters like age of the patient, menopausal state, primary tumor size and axillary lymph nodes status.

Keywords: Breast carcinomas, HER-2/neu, ER, PR

Introduction

Breast carcinoma is the most common malignant tumor and the leading cause of cancer death in women, with more than

*for correspondence: E-mail dr.nazarj@gmail.com

one million cases occurring worldwide annually⁽¹⁾. According to Iraqi Cancer Registry, breast carcinoma is the most frequent cancer among women. It forms 15.3% of all malignant tumors and 29% of the registered female cancers with the sharp increase in incidence of this tumor in young age group⁽²⁾. The average age of patients with breast carcinoma in Iraqi females is 45 years⁽³⁾.

In Arab countries, breast cancer is also the most common cancer among Egyptian, Palestinian and Kuwaiti women^(4,5).

In the USA, statistics showed that one in every eight women will develop breast cancer during their lifetime. The disease is uncommon before age of 25 years, but afterward there is a steady rise in incidence till the time of menopause; followed by a slower rise throughout life. The average age at diagnosis is 64 years⁽⁶⁾.

Prognosis and management of breast cancer are influenced by the classic variables such as histologic type and grade, tumor size, lymph node status, status of hormonal receptors—estrogen receptor (ER) and progesterone receptor (PR)—of the tumor and more recently, HER-2 status^(7,8). 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) overexpressed in 15% to 25% of invasive breast carcinomas and is associated with a worse clinical outcome^(9,10).

Immunohistochemical detection of HER-2 protein products of amplified genes through the utilization of monoclonal antibodies has become a reproducible technique⁽¹¹⁾. Through the application of the latter an associations have been found to exist between amplification and/or overexpression of HER-2/neu and a wide variety of different clinical and pathological features of breast carcinoma that include large tumor size, higher histologic grade, lack of steroid receptor expression (ER and PR), axillary lymph nodes metastasis, advanced stage, early relapse, and reduced overall survival

^(12,13). The Food and Drug Administration Agency (FDA) approved Trastuzumab (Herceptin), a monoclonal IgG1 class humanized murine antibody, for the therapy of metastatic breast cancer.

Trastuzumab is capable of blocking cell proliferation of tumor cells in both in vitro assays and experimental animals. It also induces antibody dependant cellular cytotoxicity against tumor cells. Only patients with gene amplification and/or overexpression are eligible for this treatment^(14,15). Because of these advances is, admittedly, a need to evaluate HER-2/neu status in breast cancer specimens of patients who might benefit from such a therapy.

In contrast, ER is expressed in 70% to 95% of invasive lobular carcinomas and in 70% to 80% of invasive ductal carcinomas, and PR is expressed in 60% to 70% of invasive breast carcinomas⁽¹⁶⁾. Expression of ER and/or PR (detected also by immunohistochemical method) generally is associated with a better outcome. Survival and response to hormone therapy (tamoxifen) 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^(17,18). 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 overexpressing HER-2 do not respond to tamoxifen therapy⁽⁹⁾. Although HER-2 expression generally is inversely correlated with ER and PR expression,^(19,20) the precise extent of its inverse relationship and its association with clinical and classic histologic prognostic indicators has not been studied systematically in Iraqi breast cancer cases.

Patients' and Methods

Forty-one specimens of formalin-fixed, paraffin embedded breast cancer tissue, collected from breast cancer patients over

a period from January 2011 through February 2012 were included in this study which was carried out at Al-Hussein medical city-karbala. Samples were either in the form modified radical mastectomy or an excisional biopsy followed by mastectomy. The sources of these materials were the histopathology department of Al-Hussein medical city lab. and one of the private labs. (Al-Sajjad private histopathology laboratory).

The relevant clinical data were obtained by reviewing the medical files of the patients, which included information regarding age as well as, tumor size and axillary lymph nodes status.

Histologic Examination

Histologic assessment of tumor type and grade were performed routinely on 4 – 5 µm thickness H&E-stained sections (Hematoxylin and Eosin stains) of tumors according to the criteria outlined in the World Health Organization Classification of Tumours⁽²¹⁾. Histological grading according to Nottingham Modification of Bloom-Richardson Grading Scheme which is elisted by Elstone⁽²²⁾ (table 1).

This scheme result in a total score of 3 to 9 points by adding the score from tubules formation plus number of mitoses plus nuclear pleomorphism. So, if the score is 3-5 points= Grade I (well differentiated). If the score is 6-7 points= Grade II (moderately differentiated).

If the score is 8-9 points= Grade III (poorly differentiated).

Immunohistochemical Analysis

Tissue sections (4 µm thick) were used for all immunohistochemical analyses. The confirmed anti-ER and anti-PR monoclonal antibodies (DAKO) were used and performed according to the manufacturer's instructions. The ER and PR expression was 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 monoclonal anti c-

erbB2 (neu) protein antibody (IgG1, Kappa Mouse monoclonal isotype DAKO) according to the manufacturer's instructions, and results were interpreted manually following the steps of Hercep test interpretation as illustrated in table (2)

- Zero(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)

According to this scheme HER-2 was overexpressed if the immunohistochemical score was 2+ or 3+ , but only immunohistochemical score 3+ was defined as HER-2 positive ,because 2+ score defined as equivocal and Fluorescence In Situ Hybridization (FISH) analysis is recommended to detect HER-2 gen amplification and confirm the result⁽²³⁾.

Statistical analysis

Chi-square test and Fisher's exact test were used for the statistical analysis of the variables between different subgroups⁽²⁴⁾. P-value equal or less than 0.05 is considered statistically significance according to SPSS computerized system.

Results

The overall mean age of the forty- one cases of breast carcinomas was 45 years ; the range was 30-70 years and the peak age incidence was the fifth decade (40.1%).Of the forty-one breast carcinoma cases studied, thirty six cases (87.7%) were examples of invasive ductal carcinoma of not other wise specified type (NOS). The remaining five cases showed invasive lobular carcinoma (classical type) in two cases (4.8%) , two cases were medullary carcinoma (4.8%) and only one case was cribriform carcinoma(2.4%) .

According to Nottingham Modification of Bloom- Richardson grading system , the results were ;

Grade I carcinoma (well differentiated) was seen in three cases (7.3%), grade II (moderately differentiated) seen in thirty case (73.1%) and grade III (poorly differentiated) was seen in eight cases (19.5%).

Out of forty one cases, only thirty three (80.5%) has a primary tumor size equal to or more than two cm in greatest dimensions and only eight of the cases (19.5%) had a diameter below two cm. Two thirds of the cases (65.8%) shows axillary lymph node metastasis.

Generally ER expression was seen in 66% of all breast cancer cases, while PR expression was seen in 70.8% of breast cancer cases. All the cases (100%) of lobular and cribriform type ductal carcinoma were positive for ER and PR immunohistochemically, while in the thirty six case of invasive ductal carcinoma (NOS type), 24 cases (66.6%) were ER positive, and 27 case (72.1%) were positive for PR immunohistochemistry (slightly more expression of PR than ER), all the cases of medullary carcinoma were negative for ER and PR (Table -6).

However, 29% of all the cases (12/41) were negative for both ER and PR receptors. None of the cases were ER positive, PR negative, while about 4.9% were ER negative, PR positive.

From the total study group cases (n=41), nineteen case were showing HER-2/neu overexpression (score +2 or +3), 84% of them (sixteen case) were invasive ductal carcinoma (NOS type), while 16% was seen in the other breast cancer cases (lobular and special type carcinoma as a group).

HER-2 positivity (score +3) was seen only in six cases (6/41) 16.65%. Because HER-2 score +2 considered an equivocal result and it needs to be further analyzed by FISH analysis.

When HER-2 status was analyzed according to the histologic features of tumors, we found that HER-2 positivity virtually was limited to invasive ductal carcinoma of the NOS type (6/41) and all the other cancer types (lobular, medullary, and cribriform types) were negative for the immunohistochemical staining of HER-2/neu. (Table 3).

Statistical analysis revealed significant difference between HER-2/neu positivity with histopathological type 3 (P= 0.04).

Statistical analysis revealed no significant difference between HER-2/neu positivity and primary tumor size, no significant difference between HER-2/neu positive state and axillary lymph node metastasis (P= 0.63) and no significant difference regarding age and menopause state (P= 0.43).

Table 1. The Nottingham Modification of Bloom-Richardson Grading Scheme

Histological feature	Score 1	Score 2	Score 3
Tumor Tubule Formation	IF>75% of tumor cells arranged in tubules	IF>10% and <75%	IF<10%
Number of mitosis [via low power scanning(x100) locates the most mitotically active area of tumor and proceeds to high power (x400)]	IF<10 mitoses in 10 HPF	IF>10 and <20 mitoses/10 PF	IF>20 mitoses/10HPF
Nuclear Pleomorphism	IF cell nuclei are uniform in size, shape, relatively small, have dispersed chromatin pattern, and are without prominent nucleoli	Cell nuclei are somewhat pleo-morphic, have nucleoli and are of intermediate size	Cell nuclei are relatively large, have prominent nucleoli or multiple nucleoli, coarse chromatin pattern and vary in size and shape

Association of HER-2/neu positivity and ER and PR expression .The expression of ER or PR was decreased significantly in HER-2+ tumors in comparison with HER-2- tumors. However, a substantial number of HER-2+ tumors still expressed ER (16.6 %) or PR (33%)(Table 4).

No significant correlation was observed between HER-2/neu overexpression and higher histological grades of invasive ductal carcinoma (P= 0.60).

All cases of grade I invasive ductal carcinoma were negative for HER-2/neu overexpression, whereas 16% of grade II and 25% of grade III were positive. (Table 11) .Moreover there is inverse correlation of tumor histologic grades and ER and PR expression. Only 7.3 % of all our cases (3/41) were negative for expression of estrogen and progesterone receptors (ER/PR) and HER2 oncoprotein (Triple negative breast cancer).

Table 2. Hercep test scoring system.

Score	HER-2/neu protein overexpression	Staining pattern
0	Negative	No membrane staining is observed or membrane staining is observed in less than 10% of tumor cells.
1+	Negative	A faint barely perceptible membrane staining is detected in more than 10% of tumor cells. The cells are only stained in part of their membrane.
2+	equivocal	A weak to moderately intense complete membrane staining is seen in more than 10% of cells.
3+	Positive	A strong complete membrane staining is observed in more than 10% of tumor cells.

Table 3. HER-2 , ER and PR status in different tumor types.

Tumor type	Total	HER2 +	ER +	PR +
Ductal (NOS)	36	6 (16.6%)	24 (66.6%)	27 (72.1%)
Lobular	2	0 (0%)	2 (100%)	2 (100%)
Medullary	2	0 (0%)	0 (0%)	0 (0.7%)
Cribriform	1	0 (0%)	1 (100%)	1 (100%)
Total	41	6 (14%)	27 (65.8%)	29 (70.7%)

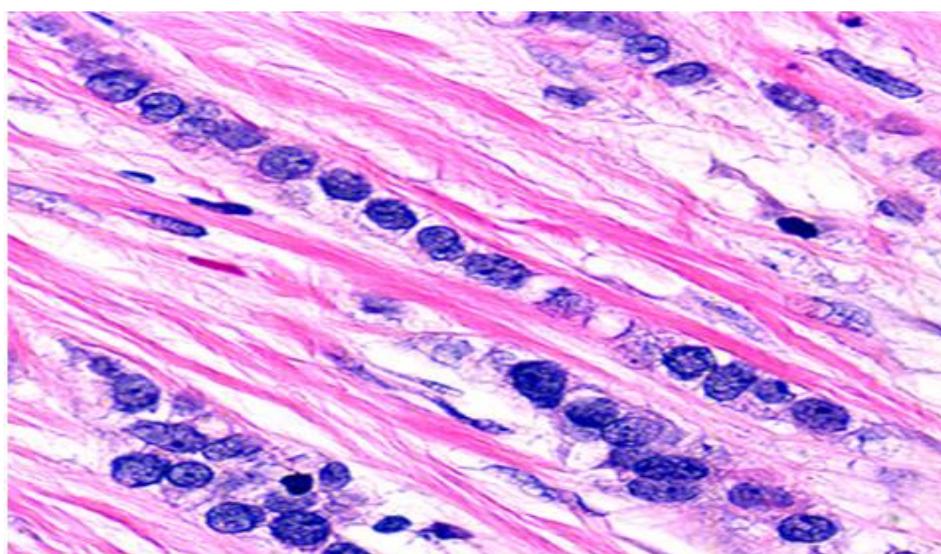


Figure 1. invasive lobular carcinoma of breast ,H&E stain,40x.

Table 4. Association of HER-2 status and ER and PR expression in all breast cancer cases (n=41)

HER -2	Total	ER +	ER -	PR +	PR -
Negative	35 (86%)	26 (74.2%)	9 (25.7%)	27 (77.1%)	8 (22.8%)
Positive	6 (14%)	1 (16.6%)	5 (83.3%)	2 (33.3%)	4 (66.6%)
Total	41 (100%)	27 (65.8%)	14 (34.1%)	29 (70.7%)	12 (29.2%)

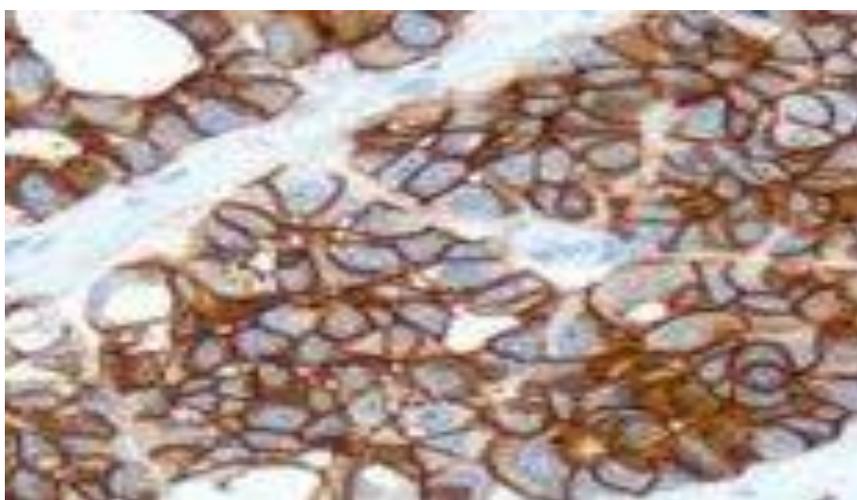
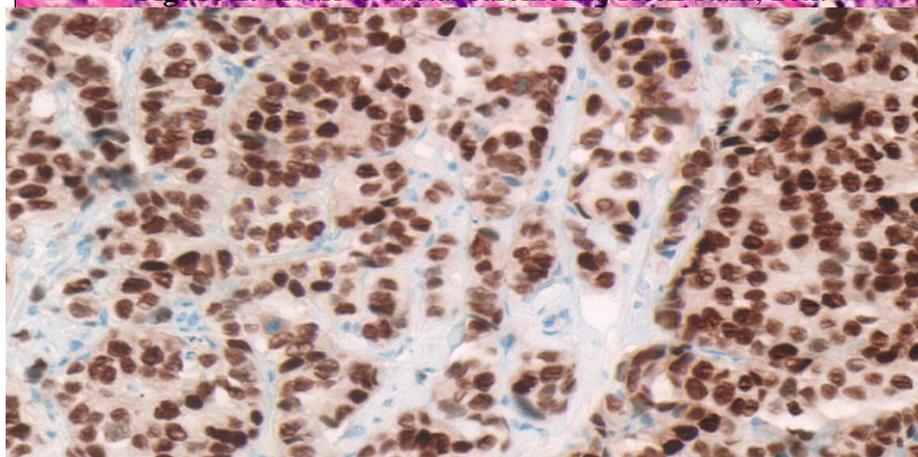
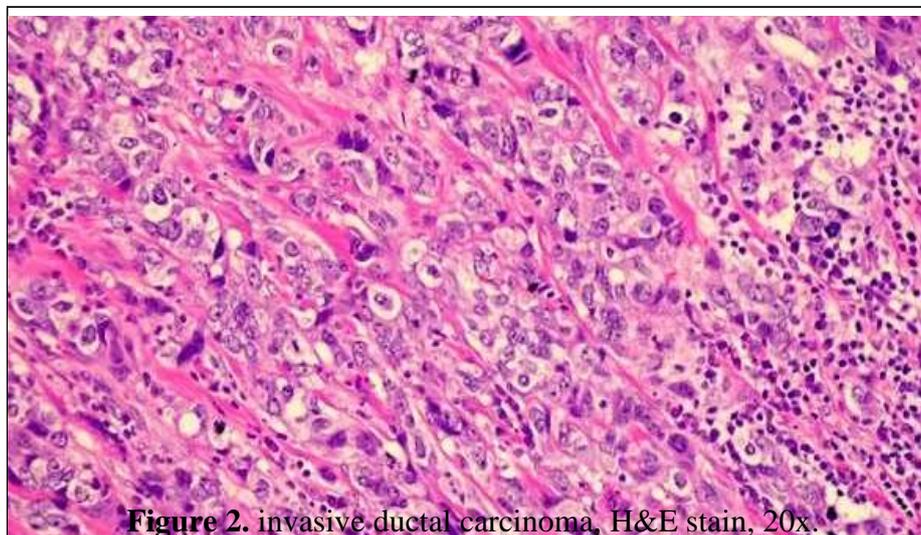


Figure 4. HER2/neu score 3, positive, in ductal carcinoma, strong and entire membrane staining, 40x.

Discussion

Breast cancer is a heterogeneous disease with variable clinical, pathological and biological characteristics. The racial influence in invasive breast cancer in terms of age at presentation, clinico-pathological features, and outcome of treatment, has been widely reported⁽²⁶⁾. It has been found that breast cancer in many Asian and African countries tends to affect younger females, presents in advanced stage, with poor prognostic features, and thus having a worse outcome in comparison with their counterparts in Western countries^(27,28). There is no doubt that the lack of understanding of the seriousness of the disease on the part of the patient and the lack of screening program may contribute to advanced presentation. However, the biological 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 proto-oncogenes may contribute to the development or progression of breast cancer⁽²⁹⁾. Alterations in the HER-2/neu expression have been studied in females with breast cancer from both Western as well as Asian countries. The outcome of these studies has been the proposal to regard HER-2/neu as a marker of prognostic significance and of potential clinical utility^(30,31). The incentive was that breast cancer in Iraq (as elsewhere) is the most common tumor in females, It forms 15.3% of all malignant tumors and 29% of the registered female cancers with the sharp increase in incidence of this tumor in young age group⁽²⁾. We performed this retrospective study to correlate HER-2 status with other routinely used tumor characteristics such as tumor type, tumor grade and hormonal status in primary breast carcinomas along with the age of the patient and axillary lymph nodes status regarding presence or absence of metastasis.

Infiltrative ductal carcinoma (NOS) is the major histological type of breast cancer among women of different races worldwide (fig 2); their rate of frequency ranges from 47% to 79% and infiltrative lobular carcinoma 2% to 15%, (Harris *et al*)⁽³²⁾.

In Iraq, according to Iraqi Cancer Registry, the incidence of IDC (invasive ductal carcinoma) was 72.0 % whereas that of ILC (invasive lobular carcinoma) (fig. 1) was 3.4% and medullary carcinoma was 0.72%⁽²⁾.

Significant correlation between HER-2/neu positivity and histopathological type was reported in this study (P= 0.04). Of the 87.7 % of the patients, in this study, who have had IDC (NOS), 46.3 % show HER-2 overexpression (score +2 or +3) and 14% , were HER-2/neu positive (score +3). This result is comparative to that reported by Priti Lal *et al* ⁽³³⁾ who reported a positive results of 15.6% , while Matloob *et al*⁽³⁴⁾, Aziz *et al* and Al-Moundhir *et al*^(35,36) reported an overexpression frequency of 41% and 39.4% and 19% respectively.

In this study, HER-2/neu was negative in all cases of infiltrative lobular carcinoma and carcinoma of special type . Although this could be due to the small number of cases of ILC , medullary and cribriform carcinomas submitted; yet, this negative result (regarding ILC) was also reported in the review of , Matloob *et al* , Vijver *et al*^(34,37) and Poter *et al*⁽³⁸⁾ in their separate studies. This high discrepancy between IDC and ILC regarding HER-2/neu overexpression has led to the alternative suggestion that this may be explained by differences between the two types at the molecular levels. In view of the above, cases of ILC and carcinoma of special type were not submitted for statistical analysis in this study.

No significant correlation was observed between HER-2/neu overexpression and higher histological grades of invasive ductal carcinoma in this study (P = 0.604)

All cases of grade I (low grade) invasive ductal carcinoma were negative for HER-2/neu overexpression, whereas 16% of grade II and 25% of grade III were positive. This is closely comparable to the results of Al-Moundhri *et al*⁽³⁶⁾ study, which revealed no statistical correlation between HER-2/neu overexpression and histological grading of the tumor, but disagree with Priti Lal *et al*⁽³³⁾ Matloob *et al*⁽³⁴⁾ and Aziz *et al*⁽³⁵⁾ which revealed significant statistical correlation between HER-2/neu overexpression and histological grading of the tumor.

Our data further demonstrated that only a minority of HER-2+ tumors were grade 2, and the rates of ER and PR expression in grade 2 tumors were significantly higher than those of grade 3 tumors.

Another finding of the current study was the lack of a correlation between lymph nodes involvement and HER-2/neu status ($P=0.36$) in patients with primary breast carcinoma. This indicates that the frequency of cancer HER-2/neu positivity is not influenced by the lymph nodes status.

Similar results were obtained with Matloob *et al*⁽³⁴⁾, Al-Moundhri *et al*⁽³⁶⁾, and Vijver *et al*⁽³⁷⁾ studies. These data strongly support the suggestion that lymph nodes involvement is a prognostic rather than a selection criterion, for adjuvant treatment⁽³⁹⁾. However, Aziz *et al*⁽³⁵⁾ study showed a strong correlation between HER-2/neu overexpression and axillary lymph nodes positivity.

Generally ER expression was seen in about 66% of all breast cancer cases, while PR expression was seen in about 70.8% of breast cancer cases. All the cases (100%) of lobular and cribriform type carcinoma were positive for ER and PR immunohistochemically. 29% of all the cases (12/41) were hormone receptors negative for both ER and PR, none of the cases were ER positive, PR negative, while about 4.9% were ER negative, PR positive. Our findings were comparable with that in literature except our results of

(ER+/PR-) were very low in comparison⁽²⁵⁾, and this may be explained by the slight more expression of PR receptors than ER expression in our study (ER 66% vs 70.8% for PR).

Our data are consistent with those of other published studies in that ER and/or PR expression in general is correlated inversely with HER-2 overexpression. However, a substantial number of HER-2+ tumors still expressed ER (16%) or PR (33%). Our results are comparative with those reported by Priti Lal *et al*⁽³³⁾ who similarly found that ER or PR expression was decreased significantly in HER-2+ tumors, a proportion of these tumors still expressed ER (49.1%) or PR (24.3%).

Only 7.3% of all our cases (3/41) were negative for expression of estrogen, progesterone receptors (ER/PR) and HER2 oncoprotein (Triple negative breast cancer). Triple-negative breast cancer is a subtype of breast cancer that is clinically negative for expression of estrogen and progesterone receptors (ER/PR) and HER2 protein⁽²⁵⁾. It is characterized by its unique molecular profile, aggressive behavior, distinct patterns of metastasis, and lack of targeted therapies. Epidemiologic studies illustrate a high prevalence of triple-negative breast cancers among younger women and those of African descent. Increasing evidence suggests that the risk factor profile differs between this subtype and the more common luminal subtypes. It is estimated that over 1 million women worldwide will be diagnosed with breast cancer, of which 15% will be classified as "triple-negative."⁽²⁵⁾

Regarding other clinical parameters, no association was found between HER-2/neu immunopositivity and patient age, menopausal state, primary tumor size. Similar results were reported by Matloob *et al*⁽³⁴⁾, Aziz *et al*⁽³⁵⁾, and Al-Moundhri *et al*⁽³⁶⁾ studies.

However, strong correlation was found between HER-2/neu overexpression and

primary tumor size in Vijver *et al* study⁽³⁷⁾. This association between HER-2/neu overexpression and large tumor size may point to a higher growth rate in tumors with HER-2/neu overexpression.

The biological activity of HER-2/neu is conditioned not only by its abundance but also by the availability of autocrine and paracrine growth factors in the tumor and the presence of three other related receptor tyrosine kinases that are necessary for signaling by HER-2/neu.⁽³⁵⁾

It has been suggested that measuring HER-2/neu overexpression may produce a powerful prognostic factor, providing additional and independent predictive information on both the interval to relapse and overall survival in patients with node-positive or node-negative breast carcinoma⁽³⁵⁾, but the main goal for studying HER-2/neu overexpression in invasive breast cancer is to select patients suitable for Herceptin therapy. Hopefully, in the near future HER-2/neu testing will become a routine examination for all breast cancer patients⁽³⁵⁾.

Conclusions

1. HER-2/neu protein is expressed more in ductal carcinoma (NOS) than in lobular carcinoma and carcinoma of special type (medullary and cribriform) taking in consideration the limited number of tumors diagnosed as such in this study .
2. HER-2/neu overexpression is more frequently detected in higher-grade IDC tumors and it is virtually limited to carcinomas of grades 2 and 3 .
3. Although ER or PR expression was decreased significantly in HER-2 positive tumors, a substantial proportion of these tumors still expressed ER (16%) or PR (33.3%).
4. HER-2/neu overexpression is not correlated with other clinico-pathological parameters .

Recommendations

1. Using the valuable HER-2 immunohistochemical test routinely in cases of breast cancer .
2. Increase the sample size with short and long follow up of the patients to study the correlation between HER-2/neu overexpression and survival rates.
3. Study HER-2/neu gene amplification by in situ hybridization technique to confirm the positivity in the immunohistochemically equivocal cases (HER-2 score +2) .
4. Study the relationship between HER-2/neu overexpression and other parameters like proliferative markers and effect of treatment with Herceptin.
5. Assessment of HER-2/neu overexpression in tumors other than breast cancer such as lung carcinoma.

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