 doi: 10.5146/tjpath.2010.01026

Correlation of Hormone Receptors with HER2/neu Protein Expression and the Histological Grade in Invasive Breast Cancers in a Cohort of Saudi Arabia

Suudi Arabistan Toplumunda İnvaziv Meme Kanserlerinde Hormon Reseptörlerinin HER2/neu Protein Ekspresyonu ve Histolojik Grade ile İlişkisi

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ABSTRACT

Objective: Data on hormone receptor and HER2/neu expression in breast cancers from Saudi Arabia and Gulf Region is sparse. We undertook this study to describe the patterns of hormone receptor and HER2/neu protein expression in breast carcinoma and compared them with the histological grade at a university hospital in Riyadh.

Material and Method: We conducted a retrospective hormone receptor and HER2/neu study on 164 histologically confirmed invasive ductal carcinoma of the breast between the year 2002 and 2008. Immunohistochemical analysis for estrogen receptor, progestrone receptor and HER2/neu was done in all the cases. Fluorescent in situ hybridization (FISH) for HER2/neu gene amplification was performed in all 2+ cases and a few equivocal 1+ and 3+cases by immunohistochemistry. The results were then compared in a blinded fashion. Correlation between Estrogen Receptor, Progesterone Receptor and HER2/neu amplification and grade of tumour were calculated.

Result: The prevalence of estrogen receptor, progestrone receptor and HER2/neu overexpression were 64.6%, 57.3% and 35.3% respectively. The expression of estrogen receptor and progestrone receptor were significantly correlated ($p<0.001$). There was also a significant negative correlation between expression of hormone receptor and HER2/neu amplification. The histological grade of the tumour was also significantly correlated to the expression of both estrogen receptor and progestrone receptor. However, the relationship between HER2/neu amplification and grade of tumour was not significant and many of the grade III tumours were HER2/neu negative. In addition, HER2/neu gene amplification by FISH was observed in 84.6% of breast cancers that were 3+ and in 18.75% of the cases that were 2+ by immunohistochemistry.

Conclusion: The revalence of estrogen receptor, progestrone receptor expression and HER2/neu amplification in breast cancers in Saudi Arabian population is similar to that reported internationally. There is a negative correlation between hormone receptors expression and HER2/neu amplification. However, not all high-grade breast cancers showed HER2/neu positive status.

Key Words: Breast cancer, HER2/neu, Estrogen receptor, Progesterone receptor

ÖZ

Amaç: Suudi Arabistan ve Körfez bölgesinde meme kanserlerinde hormon reseptörü ve HER2/neu ekspresyonu ile ilgili veriler azdır. Bu çalışmayı Riyad'da bir üniversite hastanesinde meme kanserlerinde hormon reseptör paternlerini ve HER2/neu protein ekspresyonunu tanımlamak üzere ele aldık ve bunları histolojik grade ile karşılaştırdık

Gereç ve Yöntem: 2002 ve 2008 yılları arasında, histolojik olarak tanısı doğrulanmış 164 meme invaziv duktal karsinoma olgusunda retrospektif olarak hormon reseptörü ve HER2/neu çalışması yaptık. Östrojen reseptörü, progesteron reseptörü ve HER2/neu için immünohistokimyasal analiz tüm olgularda değerlendirildi. İmmünohistokimyasal olarak tüm +2 olgulara ve az sayıdaki şüpheli +1 ve +3 olgulara HER2/neu gen amplifikasyonu için floresan in situ hibridizasyon uygulandı. Daha sonra sonuçlar kör yöntem ile karşılaştırıldı. Östrojen reseptörü, progesteron reseptörü ve HER2/neu ampilifikasyonu ve tümör grade'i arasındaki ilişki değerlendirildi.

Bulgular: Östrojen reseptörü, progesteron reseptörü, HER2/neu aşırı ekspresyonunun prevalansı sırasıyla %64.6, %57.3 ve % 35.3 idi. Östrojen reseptörü ve progesteron reseptörü ekspresyonu anlamlı derecede ilişkiliydi ($p<0.001$). Hormon reseptör ekspresyonu ve HER2/neu amplifikasyonu arasında anlamlı derecede negatif ilişki saptandı. Tümörün histolojik grade'i aynı zamanda hem östrojen reseptörü hem de progesteron reseptörü ekspresyonu ile anlamlı derecede ilişkili bulundu. Bununla birlikte, HER2/neu ampilifikasyonu ve tümör grade'i arasında anlamlı ilişki yoktu ve grade III tümörlerin birçoğu HER2/neu negatifti. Ayrıca; immünohistokimyasal olarak +3 olan meme kanserlerinin %84.6'sında ve +2 olguların %18.75'inde FISH ile HER2/neu gen ampilifikasyonu gözlendi.

Sonuç: Suudi Arabistan toplumundaki meme kanserlerinde östrojen reseptörü, progesteron reseptörü ekspresyonu ve HER2/neu amplifikasyonu ilişkisi uluslararası literatürde bildirilenler ile benzerdir. Hormon reseptörlerinin ekspresyonu ile HER2/neu amplifikasyonu arasında negatif bir ilişki vardır. Bununla birlikte tüm yüksek grade'li meme kanserleri HER2/neu pozitifliği göstermemektedir.

Anahtar Sözcükler: Meme kanseri, HER2/neu, Östrojen reseptörü, Progesteron reseptörü

Received : 14.04.2010
Accepted : 15.06.2010

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INTRODUCTION

Breast cancer is the commonest malignancy of females all over the world and the second leading cause of death due to malignancy among females. There is a variation in its incidence among multicultural populations, which suggests that etiologic factors differ in their biological expression and impact on the disease outcome. The prognosis and management of breast cancer is influenced by classic variables as the histological type, grade and stage, status of estrogen receptor (ER) and progestrone receptor (PR) and more recently, HER2/neu status (1).

The expression of ER is seen in 70-80% and PR in 60-70% of cases of invasive ductal carcinoma (2). These cases are associated with better outcome and good response to hormone therapy both in adjuvant and metastatic settings. HER2/neu, also called ErbB2, is a member of human epidermal growth factor family and encodes a transmembrane tyrosine kinase receptor. It is a proto-oncogene which is located on chromosome 17, and is amplified or the protein HER2/neu overexpressed in 15-25% cases of breast cancer (3). This marker has been extensively studied and it has been shown that HER2/neu is a significant prognostic marker in both node positive and in node negative breast cancer patients (4). The positivity of HER2/neu in breast cancer is associated with high histological grade and tumour aggressiveness. It is thus assessed for both the prognosis and prediction of response to trastuzumab treatment, chemotherapy and endocrine therapy.

Immunohistochemistry (IHC) has become the method of choice for ER and PR assessment in the last two decades. The most common cutoff to define ER positivity is 10% but there is convincing data that patients with even 1% ER positive tumour cells benefit from hormone therapy (5). Regarding the HER2/neu status, there is no agreement as to the best method of assessing amplification and over-expression as no standardised validated method exists for HER2/neu testing. The Food and Drug Administration (FDA) has approved the Hercep Test for IHC and PathVysion for fluorescent in situ hybridization. These are the most commonly used methods for HER2/neu assessment nowadays as well as in our present study.

The aim of this study was to demonstrate the pattern of expression of hormone receptor and HER2/neu in breast carcinoma, the correlation between histological grade of the lesion, and ER and PR hormonal status and HER2/neu expression. Cases equivocal for HER2/neu expression by IHC were resolved by FISH analysis.

MATERIAL and METHOD

This was a retrospective study. We studied 164 patients, histologically confirmed as invasive ductal carcinoma of the breast, who underwent total mastectomy or lumpectomy with or without axillary dissection between 2002 and 2008 at King Khalid University Hospital. The formalin fixed, paraffin embedded 4-5 micrometer thick sections were stained by routine haematoxylin and eosin stain and the histological assessment for the grade was done using the modified criteria of Bloom & Richardson Scoring System.

ER/PR analysis was carried out in all 164 cases according to the manufacturer's instruction as a routine procedure on 4-micrometer sections of formalin fixed, paraffin embedded tumour after microwave antigen retrieval (0.01M Citrate buffer, pH 6.0), using commercially available mouse monoclonal antibodies to ER and PR (clone 6F11 and Clone 16 respectively, Ventana, AZ, USA). The staining for ER and PR was classified as positive if more than 10% of the tumour cells exhibited nuclear staining. Her-2/ neu immunohistochemical analysis was performed using the Hercep Test kit (clone CB11, Dako, CA, USA) according to the manufacturer's instruction and the results were interpreted using the original FDA and new ASCO/CAP guideline recommendations (6). Scoring was done on a 0-3 scale. Positive (3+) was defined as strong complete membranous staining in more than 30% of the tumour cell population. Borderline (2+) was defined as moderate membranous staining in more than 10% of tumour cells. 1+ was defined as either weak or barely perceptible membranous staining in more than 10% of the tumour cells. 0 was completely negative staining or membranous staining in less than 10% of the tumour cells. Scores of 0 and 1+ were considered as negative for HER2/neu expression, 3+ as immunopositive while 2+ was weakly or borderline positive. Positive and negative controls were included in each batch.

FISH analysis was performed for HER2/neu gene amplification in all 2+ cases (32 cases) by IHC, and 28 equivocal cases of 1+ and 3+ (showing some technical fault as staining of the blood vessel or benign ducts) between 2005 and 2008. The cases were sent to Kassel Klinikum in Germany (as this test is not available in our institution) for further assessment by FISH analysis to confirm or exclude gene amplification. HER2/neu gene amplification was determined in paraffin-embedded tissue sections by FISH using PathVysion (Abbott/Vysis, IL, USA) as a ratio of HER2/neu gene copies-to chromosome 17 centromere copies.

Statistical Methods

Statistical significance was set at $p < 0.05$. Data were analyzed by the chi-square test. Computations were performed with the sigma STAT for Windows software (version 2.03, SPSS Inc., IL, USA).

RESULTS

Of the total 164 cases, 108 expressed ER, PR, or both (ninety two cases expressed both ER and PR, fourteen cases only ER and two cases only PR) (Figure 1, 2). The remaining 56 cases were both ER and PR negative. Patients were thus divided into the following four groups: ER + /PR+ (92/164, 56%), ER+/PR- (14/164, 8.5%), ER-/PR+ (2/164, 1.2%) &ER-/PR- (56/164, 34.2%) as shown in Table I. A statistically significant correlation was found between the expression of ER and PR ($p < 0.001$).

As regards the HER2/neu expression by IHC, 58 cases were 3+ (Figure 3), 23 cases were 2+ (Figure 4) and the remaining were either 1+ or 0 , out of the total of 164 cases,. It was found that ER and PR expression was increased significantly in HER2/neu negative tumours compared to Her-2 positive tumours. However, a substantial number of Her-2 positive tumours still expressed ER or PR (Table II). Thus there was a statistically significant negative correlation between the expression of hormone receptor and HER2/neu overexpression.

Out of 164 cases of invasive ductal carcinoma of the breast, 9 cases were grade I, 85 cases were grade II and 70 cases were

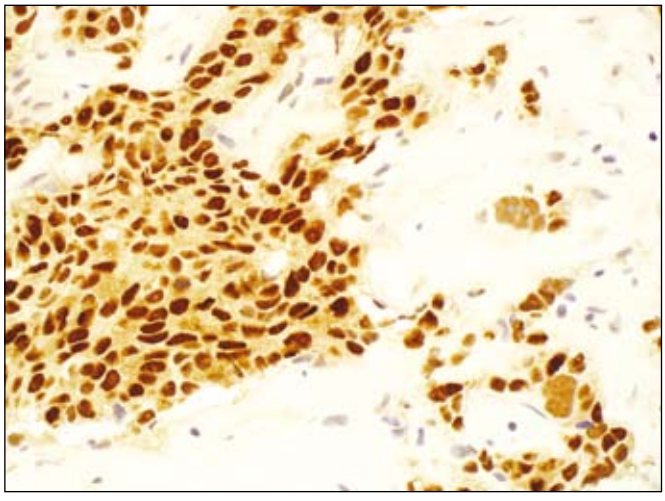

Figure 1: IHC showing estrogen receptor positive tumour cells in breast cancer (x400).

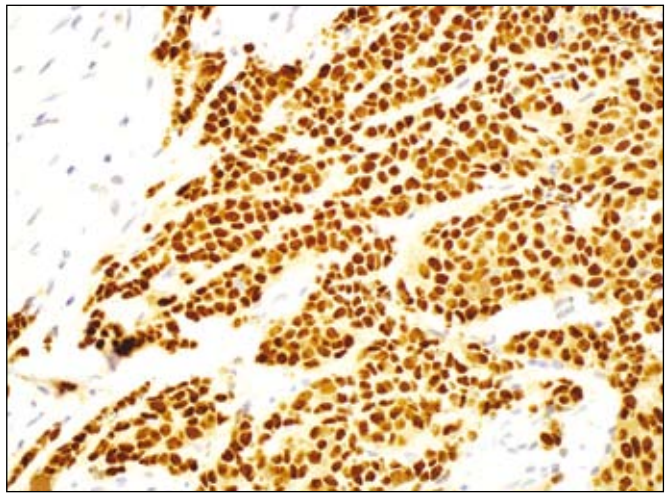

Figure 2: IHC showing progesterone receptor positive tumour cells in breast cancer (x400).

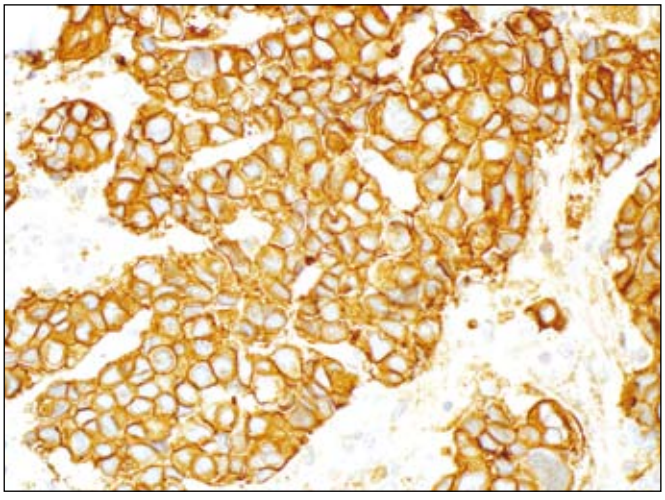

Figure 3: HER2/neu immunostaining: strongly positive (3+) pattern showing intense membranous staining in more than 10% of the tumour cells (x600).

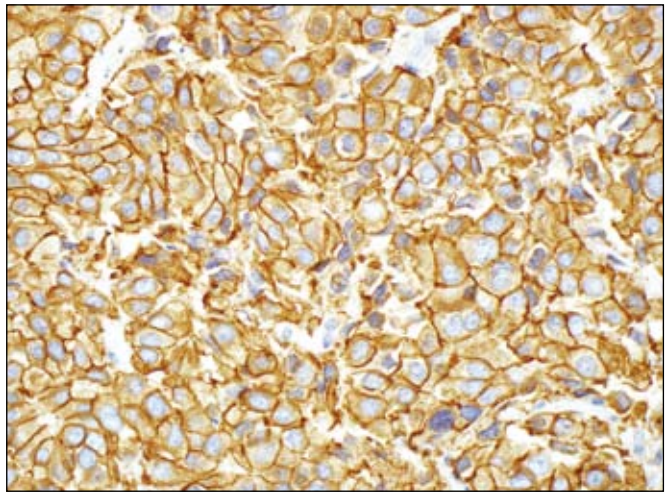

Figure 4: HER2/neu immunostaining: intermediate (2+) pattern showing moderately complete membranous staining in more than 10% of the tumour cells (x600).

grade III. The reactivity for steroid receptors was observed to decrease with increasing grade. Grade II tumours expressed ER and PR more than grade III tumours (Table III). Thus the grade of tumour was significantly correlated to the expression of both ER and PR (Pearson chi-square value = 6.085 and 11.169 respectively with p values of 0.047 and 0.025). The relationship between HER2/neu overexpression and grade of tumour was found to be not significant (p value > 0.25) and vast majority of HER2/neu negative tumours were grade III.

Sixty cases out of 164 (those that were either 2+ and a few 1+ or 3+) were confirmed by FISH analysis (Table IV). The cases assigned by IHC as suspicious for 1+ (two cases) group were HER2/neu negative by FISH analysis. Among the samples described immunohistochemically as 2+ (32 cases), amplification of HER2/neu gene was discovered in 18.25% of the cases. (n=6). The remaining 81.25% of the cases (n=26) were negative by the FISH method.

In the group of specimen assessed by IHC as equivocal for 3+ (26 cases), HER2/neu gene amplification was detected in 84.6% (n=22). Lack of gene amplification by FISH was seen in 4 cases (15.3%).

Table I: Relationship between estrogen receptor and progesterone receptor

	PR +	PR -	Total
ER +	92 (56%)	14 (8.5%)	106
ER -	2 (1.2%)	56 (34.2%)	58
Total	94	70	164

DISCUSSION

ER, PR and HER2/neu represent the most acceptable factors for predicting prognosis, response or resistance to treatment and the potential use of newer drugs such as Trastuzumab in the case of HER2/neu overexpression.

We performed a retrospective study in a cohort of 164 patients to correlate hormone receptor and HER2/neu status with histological grade of the lesion and ER, PR hormonal status. Consistent with most widely adapted clinical practice, and the practice at our institution, we considered a tumour as HER2/neu positive as being either

Table II: Association between HER2/neu status and estrogen and progesterone receptor expression

	HER2/neu +3	HER2/neu +2	HER2/neu Negative (Score of 1+ or 0)	Total
ER+, PR+	22	26	44	92
ER+, PR-	6	2	6	14
ER- , PR+	2	0	0	2
ER- , PR-	28	4	24	56
Total	58	32	74	164

*__ER:__ Estrogen Receptor, __PR:__ Progesterone Receptor, __+:__ Positive, __-:__ Negative

Table III: Relationship of tumour grade with hormone receptor expression and HER2/neu amplification in infiltrating ductal carcinoma specimens.

	Score	Grade I (n=9)	Grade II (n=85)	Grade III (n=70)
Her-2 neu	3+	0	28	30
	2+	1	18	5
	0/1+	8	40	35
ER	Negative	1	25	32
	Positive	8	60	38
PR	Negative	2	31	36
	Positive	7	54	34

Table IV: Comparison of HER2/neu status determined by FISH and immunohistochemistry

	Immunohistochemistry scores of HER2/neu			Total
	1+	2+	3+	
FISH – (%)	2 (100%)	26 (81.25%)	4 (15.3%)	32 (53.3%)
FISH + (%)	0 (0%)	6 (18.75%)	22 (84.6%)	28 (46.6%)
Total	2 (100%)	32 (100%)	26 (100%)	60 (100%)

IHC score of 3+ or FISH positive (ratio of more than or equal to 2.2).

In our study, the prevalence of ER, PR expression and HER2/neu amplification were 64.6%, 57.3%, and 35.3% respectively. These fall within the ranges given in the English literature (7-10). ER and PR determination are established procedures in the routine management of patients with breast cancer, primarily as a predictive factors for response to therapeutic and adjuvant hormonal therapy (11,12). In the published literature (13) approximately 50% of tumours are ER +, PR +, 25% ER -, PR –, 20% ER +, PR- and 5% ER –, PR +. Our study reports a lower proportion of ER +, PR- (8.5%) and a higher proportion (34.2%) of hormone receptor negative cases. Desai et al. (14) and Dutta et al. (15) also found a high incidence of steroid non-reactivity in breast cancer patients. In general, there was a statistically significant positive correlation between the expression of ER and PR in our study, as reported by others (8,16,17). This also abides by the theory of ER-dependent PR synthesis (15).

The co-expression of hormone receptors with that of HER2/neu is infrequent as previously reported. In our study, HER2/neu Receptor revealed a significant inverse association with hormone receptor status. We found that ER and PR expression was increased significantly in HER2/neu negative tumours, compared to HER2/neu positive tumours. Similar results were shown in previous studies (7,8,18-20). The generally accepted inverse correlation between the expressions of hormone receptors and HER2/neu amplification is explained by hormone-dependent downregulation of HER2/neu involving a complex molecular interaction as reviewed by Ciocca et al (21) and as shown by in vitro studies (18,19). This is one of the reasons why women who overexpress HER2/neu may be resistant to tamoxifen. However, as demonstrated in our study and by others, ER positive cases can have HER2/neu overexpression/amplification. Both ER and HER2/neu positive tumours have poorer disease-free and overall survival than ER positive, HER2/neu negative tumours, suggesting that HER2/neu overexpression may be better predictor of response to tamoxifen therapy than ER status alone (22).

The grade of tumour in our study was significantly correlated to the expression of both ER and PR (p value of 0.04 and 0.025, respectively). The lower grade tumours showed high ER and PR positivity, similar to other studies (8,23). The overexpression/amplification of HER2/neu in different histological grades of invasive ductal carcinoma of the breast has been a subject of interest. In our study, the relationship between HER2/neu amplification and grade of tumour was found to be not significant and vast majority of HER2/neu negative tumours were grade III. This is in contrast with previous studies (20,24-26), which reported histologically high-grade tumours' association with increased rate of HER2/neu overexpression.

We confirmed the results of HER2/neu protein and overexpression of all 2+ and equivocal 1+ and 3+ cases on IHC, using FISH analysis in total of 60 cases. As only patients with HER2/neu protein overexpression and/or gene amplification benefit from Trastuzumab treatment, our aim was to identify those cases, using a more accurate and reliable method – i.e. FISH. Our results indicate that the IHC 2+ group is characterized by high heterogeneity as only 18.75% of the cases showed HER2/neu gene amplification. In samples described by IHC as 3+, amplification of HER2/neu gene was discovered in 84.6% of the cases by FISH analysis. These results are in accordance with many previous reports (27-31) in the evaluation of HER2/neu status of breast cancer using formalin-fixed paraffin-embedded specimens, which found a high degree of correlation between IHC 3+ staining and amplification detected by FISH.

In conclusion, the prevalence of ER, PR and HER2/neu amplification among Saudi Arabian breast cancer patients is similar to the rest of the world. There is also a negative correlation between expression of hormone receptor and HER2/neu expression. The lower grade of tumour is related to hormone receptor expression in accordance to that reported in the literature, however not all our high-grade tumours were HER2/neu positive.

ACKNOWLEDGEMENT

I thank Dr Shaesta Naseem Zaidi, MD for her assistance in the study and preparation of the manuscript. A special thanks to Rouch Company who supported performing the FISH analysis at Kassel Klinikum in Germany.

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