OTJHS
Online Turkish Journal of Health Sciences

e-ISSN: 2459-1467

OTSBD
Online Türk Sağlık Bilimleri Dergisi

Online Turkish Journal of Health Sciences 2023;8(1):101-107 *Online Türk Sağlık Bilimleri Dergisi 2023;8(1):101-107*

Evaluation of the Relationships between ER, PR, c-erbB2, Ki67, E-Cadherin Expressions, Nottingham Histological Grade and some Clinical Parameters in Breast Carcinomas

Meme Karsinomlarında ER, PR, c-erbB2, Ki67, E-Cadherin Ekspresyonları, Nottingham Histolojik Grade ve Bazı Klinik Parametreler Arasındaki İlişkilerin Değerlendirilmesi

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ABSTRACT

Objective: In this study, it was aimed to evaluate the relationships between Estrogen receptor (ER), Progesterone receptor (PR), c-erbB2 (HER2), Ki67, E-Cadherin expressions, Nottingham histological grade and some clinical parameters in breast carcinomas.

Materials and Methods: A total of 74 patients diagnosed with breast carcinoma (CA) in our pathology department between 2018-2019 were included in this study. Immunohistochemical preparations stained with ER, PR, HER2, Ki67 and E-Cadherin were evaluated and analyzed retrospectively. For ER and PR, ≥1% expression was considered as positive staining, and <1% was considered as negative staining. HER2 expression was scored as 0, 1, 2 and 3. Ki67 proliferation index was considered as low (<10%), intermediate (10-20%) and high risk (>20%). The data were analyzed with chi-square test.

Results: HER2 score showed a statistically significant change according to ER status (p=0.010). HER2 score also showed a statistically significant change according to PR status (p=0.004). There was a significant correlation between Ki67 and histological stage (p<0.001).

Conclusions: Detection of high Ki67 index in breast carcinomas is poor prognostic. Detection of ER and PR expression and no expression of HER2 are good prognostic indicators. Preanalytical and analytical processes should be followed meticulously by pathologists.

Keywords: Breast Carcinoma, E-Cadherin, HER2, Hormone Receptors, Ki67

ÖZ

Amaç: Bu çalışmada meme karsinomlarında Östrojen (ER), Progesteron (PR), c-erbB2 (HER2), Ki67, E-Cadherin ekspresyonları, Nottingham histolojik grade ve bazı klinik parametreler arasındaki ilişkilerin değerlendirilmesi amaçlanmıştır.

Materyal ve Metot: Bu çalışmaya 2018-2019 yıllarında patoloji bölümümüzde meme karsinom tanısı alan toplam 74 hasta dahil edildi. İmmünohistokimyasal olarak çalışılan ER, PR, HER2, Ki67, E-Cadherin boyalı preparatlar retrospektif olarak değerlendirilip incelendi. ER ve PR için ≥%1 ekspresyon pozitif boyanma, <%1 ise negatif boyanma olarak kabul edildi. HER2 skor 0, 1, 2 veya 3 olarak değerlendirildi. Ki67 proliferasyon indeksi için <%10, % 10-20, >%20 sırasıyla düşük, orta ve yüksek riskli olarak kabul edildi. Çalışmada elde edilen veriler ki-kare testi ile değerlendirildi.

Bulgular: HER2 skoru ER durumuna göre istatistiksel olarak anlamlı değişim gösterdi (p=0,010). HER2 skoru PR durumuna göre de istatistiksel olarak anlamlı değişim gösterdi (p=0,004). Ki67 ile histolojik evre arasında anlamlı ilişki vardı (p<0.001).

Sonuç: Meme karsinomlarında Ki67 indeksinin yüksek olması kötü prognostik göstergelerdendir. ER, PR ekspresyonunun saptanması ve HER2 ekspresyonunun saptanmaması ise iyi prognostik göstergelerdendir. Preanalitik ve analitik süreçler patologlar tarafından titizlikle takip edilmelidir.

Anahtar Kelimeler: E-Cadherin, HER2, hormon reseptörleri, Ki67, meme karsinomu

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Yayın Bilgisi / Article Info:
Gönderi Tarihi/ Received: 04/11/2022
Kabul Tarihi/ Accepted: 11/12/2022
Online Yayın Tarihi/ Published: 05/03/2023

Atıf / Cited: Akçay Çelik M and Kaşko Arıcı Y. Evaluation of the Relationships between ER, PR, c-erbB2, Ki67, E-Cadherin Expressions, Nottingham Histological Grade and some Clinical Parameters in Breast Carcinomas. *Online Türk Sağlık Bilimleri Dergisi* 2023;8(1):101-107. doi: 10.26453/otjhs.1199237

INTRODUCTION

Mortality of breast cancer has decreased significantly in recent years with the development of new treatment options in terms of early diagnosis, surgery and oncology.[1] Grading has independent prognostic significance in breast cancer, and the Nottingham histological grade method has also been found to improve interobserver agreement compared to other grading systems.[2,3]

Histological findings and immunohistochemical (IHC) evaluations are very important in the diagnosis of breast carcinomas. IHC is a technique currently used to measure the level of Estrogen (ER) and Progesterone (PR) biomarker expression in breast cancer tissues and to evaluate the cancer response to endocrine therapy.[4,5] About 75% of breast cancers are ER-positive. The predictive and prognostic roles of PR alone in breast cancer are unclear. The ER-negative and PR-positive subgroups have been reported as 1-5% of all breast cancers. In other words, the ER-negative PR-positive cases are rare.[6,7] There are significant differences in how different laboratories perform ER and PR tests and interpret the results.[8] The c-erbB2 (HER2) is overexpressed and/or amplified in almost 15% of breast cancers. HER2-positive status is an unfavorable prognostic factor.[9,10]

Ki-67 is a proliferation marker evaluated immunohistochemically.[11] The mitotic rate is routinely estimated by the Ki67 value.[12] Ki67 provides independent predictive and prognostic benefits in chemotherapy response in adjuvant and neoadjuvant settings.[13]

Loss of tumor suppressive properties of E-cadherin is thought to be associated with invasion and carcinogenesis.[14,15] Loss of E-cadherin expression is generally used to determine lobular morphology, which accounts for 10-20% of all breast cancers.[16,17]

In our study, the evaluation of the relationships between ER, PR, HER2, Ki67, E-Cadherin expressions, Nottingham histological grade and some clinical parameters in breast carcinomas were examined.

MATERIALS AND METHODS

Ethics Committee Approval: Before starting the study, permission was obtained from the Clinical Research Ethics Committee (Date:2020; decision no:169). The study was performed according to the Declaration of Helsinki.

Study Design: The study was planned as a retrospective cross-sectional study and was conducted between 2018 and 2020.

Data Collection: This study included 74 patients diagnosed with breast carcinoma between 2018-2019 in the Department of Pathology, Faculty of Medicine, Ordu University. Pathology preparations were obtained from the archive for evaluation.

Histological Analysis and Evaluation: Cases with breast carcinoma were evaluated and analyzed retrospectively according to their ER, PR, HER2, Ki67, E-Cadherin expressions and Nottingham histological grades. In addition, some clinical parameters such as age and gender were also evaluated in these cases.

Immunohistochemically, ≥1% nuclear expression was accepted as positive staining for ER and PR, and <1% nuclear expression was considered as negative staining.[18]

HER2 score was evaluated as 0, 1, 2, and 3. (score 0: no staining or incomplete pale staining ≤ 10% in invasive tumor cells, score 1: incomplete pale membrane staining in more than 10% of invasive tumor cells, score 2: weak to moderate complete membrane staining in more than 10% of invasive tumor cells, score 3: complete, intense circumferential membranous staining (strong positive staining) in more than 10% of invasive tumor cells).[9]

Ki67 proliferation index was considered as low (<10%), intermediate (10-20%) and high risk (>20%).[19]

For E-Cadherin, membranous staining in the neoplasm was considered as positive staining.[20] The Nottingham Histologic grading system was used to grade the tumors. Tumors were graded as grade 1, 2, or 3 according to tubule or gland formation, nuclear pleomorphism, and the number of mitoses. Grade 1 was specified as having a good prognosis, Grade 2 was an intermediate prognosis and Grade 3 was the worst prognosis.[2]

Statistical Analysis: Categorical data were expressed as frequency (n) and percentage (%). Pearson's chi-square test was used to determine the relationship between the categorical variables. A continuous variable is defined as mean±standard deviation (SD). In the chi-square test, when the expected cell frequencies fall below 5, a Likelihood ratio test statistic value was calculated instead of Pearson's test statistic value. Kendall's tau correlation coefficient was calculated to measure the ordinal association. A p-value of less than 0.05 was considered statistically significant. All statistical analyses were performed using the SPSS v28 (IBM Inc., Chicago, IL, USA) statistical software.

RESULTS

There were 74 cases diagnosed with breast carcinoma in the study. There were 98.6% female and 1.6% male patients, and the mean age was 56.42±14.42 years. Half of the patients were 55 years or older, 10.8% of the remaining patients were <40 years, and 39.2% were between 40-54 years (Table 1). Invasive ductal carcinoma was found to be the most common histological type (82.4%). In our study, Tru-cut bi-

opsy material was the most common sampling method (67.7%). The localizations of malignant neoplasms were determined as 50% right, 48.6% left, and 1.4% right+ left breast localization (Table 1).

HER2 score showed a statistically significant change according to ER status (p=0.010). While the rate of HER2 score 3 was higher in ER-negative patients (72.2%), the rate of HER2 score 0 in ER-positive patients was higher (34.5%). HER2 score also showed a statistically significant change according to PR status (p=0.004). Likewise, while the rate of HER2 score 3 was higher in PR-negative patients (69.6%), the rate of HER2 score 0 in PR-positive patients was higher (36.0%) (Table 2).

Table 1. Main characteristics of patients.

		n (%)
Gender	Female	73 (98.6)
	Male	1 (1.4)
Age (Mean±SD: 6.42±14.42)	<40	8 (10.8)
	40-54	29 (39.2)
	≥55	37 (50.0)
Tumor Localization	Right	37 (50.0)
	Left	36 (48.6)
	Right&Left	1 (1.4)
Diagnosis	Ductal carcinoma in situ	1 (1.4)
	Encapsular papillary and solid papillary carcinoma	1 (1.4)
	Intraductal papillary carcinoma	1 (1.4)
	Invasive ductal carcinoma	61 (82.4)
	Invasive lobular carcinoma	4 (5.4)
	Medullary invasive breast carcinoma	1 (1.4)
	Metaplastic carcinoma	1 (1.4)
	Mixed breast carcinoma (invasive lobular, invasive ductal carcinoma)	3 (4.1)
	Solid papillary carcinoma	1 (1.4)
HER2 score	0	20 (27)
	1	13 (17.6)
	2	10 (13.5)
	3	30 (40.5)
	Not calculated	1 (1.4)
Ki67 Status	Low risk	9 (12.2)
	Moderate risk	32 (43.2)
	High risk	32 (43.2)
	Not calculated	1 (1.4)
E-Cadherin	IHK not studied	39 (52.7)
	Negative	4 (5.4)
	Positive	31 (41.9)
Histological grade	Grade 1	12 (16.2)
	Grade 2	52 (70.3)
	Grade 3	7 (9.5)
	Not graded	3 (4.1)
ER	Negative (<1%)	18 (24.3)
	Positive (≥1%)	55 (74.3)
	Not calculated	1 (1.4)
PR	Negative (<1%)	23 (31.1)
	Positive (≥1%)	50 (67.6)
	Not calculated	1 (1.4)

Ki-67: Proliferation marker; HER2: Human epidermal growth factor receptor 2; ER: Estrogen; PR: Progesterone.

Table 2. Relationship between HER2 score and Hormone receptors.

		HER2 score				Total n (%)	p, χ^2
		0 n (%)	1 n (%)	2 n (%)	3 n (%)		
ER Status	Negative	1 (5.6)	2 (11.1)	2 (11.1)	13 (72.2)	18 (100.0)	p=0.010 χ^2=11.382
	Positive	19 (34.5)	11 (20.0)	8 (14.5)	17 (30.9)	55 (100.0)	
	Total	20 (27.4)	13 (17.8)	10 (13.7)	30 (41.1)	73 (100.0)	
PR Status	Negative	2 (8.7)	2 (8.7)	3 (13.0)	16 (69.6)	23 (100.0)	p=0.004 χ^2=13.134
	Positive	18 (36.0)	11 (22.0)	7 (14.0)	14 (28.0)	50 (100.0)	
	Total	20 (27.4)	13 (17.8)	10 (13.7)	30 (41.1)	73 (100.0)	

HER2: Human epidermal growth factor receptor 2; ER: Estrogen; PR: Progesterone; p: < 0.05 significant value; χ^2: Likelihood ratio chi-square test.

Ki67 was found to be high risk at a rate of 72.2% in ER receptor-negative patients (p=0.005) and 65.2% in PR receptor-negative patients (p=0.033) (Table 3).

Ki67 did not differ significantly according to age groups (p=0.342) and HER2 scores (p=0.389). There was a statistically significant correlation between Ki67 and the histological stage (p<0.001). As Ki67 increased, the histological stage was increased (r=0.349) (Table 4).

Table 3. Relationship between Ki67 and hormone receptors.

		Ki67 Status			Total n (%)	p, χ^2
		Low n (%)	Moderate n (%)	High n (%)		
ER Status	Negative	0 (0.0)	5 (27.8)	13 (72.2)	18 (100.0)	p=0.005 χ^2=10.580
	Positive	9 (16.4)	27 (49.1)	19 (34.5)	55 (100.0)	
	Total	9 (12.3)	32 (43.8)	32 (43.8)	73 (100.0)	
PR Status	Negative	1 (4.3)	7 (30.4)	15 (65.2)	23 (100.0)	p=0.033 χ^2=6.836
	Positive	8 (16.0)	25 (50.0)	17 (34.0)	50 (100.0)	
	Total	9 (12.3)	32 (43.8)	32 (43.8)	73 (100.0)	

Ki-67: Proliferation marker; ER: Estrogen; PR: Progesterone; p: < 0.05 significant value; χ^2:Likelihood ratio chi-square test.

Table 4. Comparison of Ki67 with age, HER2 score, histological grade.

		Ki67 Status			Total n (%)	p, χ2 and r
		Low n (%)	Moderate n (%)	High n (%)		
Age group	<40	0 (0.0)	4 (50.0)	4 (50.0)	8 (100.0)	p=0.342 χ^2=4.504
	40-54	2 (7.1)	12 (42.9)	14 (50.0)	28 (100.0)	
	>=55	7 (18.9)	16 (43.2)	14 (37.8)	37 (100.0)	
	Total	9 (12.3)	32 (43.8)	32 (43.8)	73 (100.0)	
HER2 score	0	5 (25.0)	8 (40.0)	7 (35.0)	20 (100.0)	p=0.389 χ^2=6.312
	1	0 (0.0)	6 (46.2)	7 (53.8)	13 (100.0)	
	2	1 (10.0)	4 (40.0)	5 (50.0)	10 (100.0)	
	3	3 (10.0)	14 (46.7)	13 (43.3)	30 (100.0)	
	Total	9 (12.3)	32 (43.8)	32 (43.8)	73 (100.0)	
Histological Grade	Grade 1	1 (8.3)	10 (83.3)	1 (8.3)	12 (100.0)	p<0.001 χ^2=20.794 r:0.349
	Grade 2	8 (15.4)	21 (40.4)	23 (44.2)	52 (100.0)	
	Grade 3	0 (0.0)	0 (0.0)	7 (100.0)	7 (100.0)	
	Total	9 (12.7)	31 (43.7)	31 (43.7)	71 (100.0)	

Ki-67: Proliferation marker; HER2: Human epidermal growth factor receptor 2; ER: Estrogen; PR: Progesterone; p: < 0.05 significant value; χ^2:Likelihood ratio chi-square test; r: Kendalltau-b correlation coefficient.

DISCUSSION AND CONCLUSION

Among the histological types of breast carcinomas, the most common type is invasive ductal carcinoma with approximately 85%.[17] In our study, invasive ductal carcinoma was the most common histological type (82.4%), and invasive lobular carcinoma was the second most common (5.4%).

In our study, the most common histological grade was grade 2 (70.3%) and the second most common was grade 1 (16.2%).

High interobserver reproducibility of the Nottingham combined histological grade was demonstrated in the study of Rakha et al., the proportion of different grades in their series was found to be almost the same as in the study of Elston et al. (grade 1, 17%; grade 2, 37%, and grade 3, 46%).[2, 21]

There may be interobserver variability in the determination of histological grade. Differences between centers are usually due to differences in tissue preparation quality. Suboptimal tissue fixation negatively affects the mitotic rate. Therefore cases that grade 3 can be reported as grade 2.[21]

In a study, the independent prognostic importance of grading in breast cancer was emphasized, and it has also been reported that the Nottingham histological grade method improves interobserver agreement compared to other grading systems.[3]

In our study, the most common histological grade was found to be grade 2, and the difference in interpretation between observers can explain this situation. Ki67 showed a statistically significant change according to histological stages in our study (p<0.001). Histological grade 2 was detected as 44.2% and grade 3 as 100% in the high-risk Ki67 group.

According to Ersöz et al., a significant relationship was found as a result of comparing Ki67 with histological grade and mitotic activity.[22] According to another study, the histological grade was shown to be associated with other well-defined prognostic variables and outcomes of patients.[21] In neoadjuvant and adjuvant applications, Ki67 is an independent prognostic and predictive useful proliferation marker

for chemotherapy response.[13] High-risk detection of Ki67 in cases with grade 3 and grade 2 in our study was consistent with the literature. Determination of appropriate breast cancer treatments and prognostic outcomes depends on accurate histological classification and measurement of two major groups of biomarkers: hormone receptors and HER2.[4,10]

In most studies, ER-positive and/or PR-positive rates were found to be higher in tru-cut biopsies compared to surgical specimens. This may be due to better fixation of the tru-cut biopsy material compared to the surgical excision specimen.[23] In our study, the majority of specimens were tru-cut biopsies (67.7%); but since the evaluations were made on all specimens (mastectomy, lumpectomy, tru-cut biopsy), no comment could be made on immunohistochemical expression differences in tru-cut biopsy and other specimens. In our study, ER-negative cases were found to be 24.3%, and PR-negative cases were found to be 31.1%. False-negative results in ER and/or PR expressions are more common in IHC studies. Therefore, IHC studies should be performed with appropriate internal and external controls.

Major causes of false negative results include tissue fixation problems, tumor heterogeneity, and interpretation of positive cases at the lower end of the spectrum. Sensitive consideration is required to avoid false negative results from preanalytical issues. False-negative results may cause patients to be deprived of treatment.[24] Our ER and PR expression rates were followed in line with the literature.

In our study, HER2 score showed a statistically significant change according to ER status (p:0.010). While the rate of HER2 score 3 in ER-negative patients was high (72.2%), the rate of HER2 score 0 in ER-positive patients was higher (34.5%).

HER2 score showed a statistically significant change according to PR status (p=0.004). While the rate of HER2 score 3 in PR-negative patients was high (69.6%), the rate of HER2 score 0 in PR-positive patients was higher (36%).

Han et al. reported that HER2-negative tumors do not require retesting. It has been reported that HER2 -negative tumors include low-grade carcinomas with ER-positive and infiltrative ductal or lobular histology.[24] Our findings were also found to support the literature.

In our study, Ki67 was found to be high risk at a rate of 72.2% in ER receptor-negative patients and 65.2% in PR receptor-negative patients. In our study, Ki67 showed a statistically significant change according to histological grades.

There are studies in the literature reporting that Ki-67 expression is inversely proportional to ER, PR receptors, and directly proportional to histological grade, tumor size, axillary lymph node involvement, and vascular invasion.[25]

High-risk detection of Ki67 in ER and PR-negative cases supports the literature findings. Ki67 did not show significant change according to age groups and HER2 scores.

In our data, E-Cadherin was not studied immunohistochemically in some tumors (52.7%),

However, negative staining was observed in all invasive lobular carcinomas (4 cases) studied with E-Cadherin (5.4%), it was studied in 31 patients (41.9%) diagnosed with invasive ductal carcinoma and positive staining was found in them. According to Wasif et al., loss of E-cadherin expression is widely used to determine lobular histology, which accounts for 10-20% of all breast cancers.[17] E-Cadherin expression findings in our study support the literature.

Breast cancer is a heterogeneous disease that requires clinical skills and a multidisciplinary approach to diagnosis and treatment.[26] Reproductive factors such as parity, duration of breastfeeding and period of lifetime menstrual may be more likely to predict the risk of hormone receptor-positive disease, but may not be valid for all types of breast cancer.[27]

Interpretation of immunohistochemical staining in the presence of histological findings is important for diagnosis and treatment.[28,29] IHC plays a vital role in predicting prognosis and response to therapy. Nowadays, traditional techniques such as IHC in breast cancer are still indispensable.[28]

The relationships between the molecular status of breast cancer and the biological and clinical course of the tumor are very important. Breast cancer profiling studies will better understand the importance of the genetic structure of breast tumors.[30]

In conclusion, today, in many centers, ER, PR, HER2 and Ki67 are used as a combination of four markers to provide better predictive and prognostic value in breast cancer.

Grading has independent prognostic significance in breast cancer. Pathologists may have different interpretations in terms of evaluating immunohistochemical staining and histological grading. In order to reduce this, preanalytical and analytical processes should be followed meticulously by pathologists. Compared to other grading systems, the Nottingham histological grade method is known to improve the interobserver agreement.

It should be kept in mind that immunohistochemical technical artifacts significantly affect the treatment strategy of the patient.

In this manuscript, we wanted to mention the differences in interpretation between pathologists and the importance of immunohistochemical technical artifacts.

False-negative results may fail to administer effective treatment. False-positive results can also lead to costly, ineffective, and overtreatment. Therefore,

high-quality and reliable receptor evaluations are very important. In conclusion, in addition to histological type, many clinicopathological parameters such as tumor grade, hormone receptors and HER2 status, Ki67 proliferation index are of great importance in terms of determining the prognosis and determining the correct treatment strategy for breast cancer.

Ethics Committee Approval: Before starting the study, permission was obtained from the Clinical Research Ethics Committee (Date:2020; decision:169).

Conflict of Interest: No conflict of interest was declared by the authors.

Author Contributions: Concept – MAÇ; Supervision – MAÇ; Materials – MAÇ; Data Collection and/or Processing – MAÇ; Analysis and/ or Interpretation – YKA; Writing – MAÇ, YKA.

Peer-review: Externally peer-reviewed.

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