ORIGINAL ARTICLE

Association between Ki-67 expression and clinicopathological features in prognosis of breast cancer: A retrospective cohort study

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Background: Breast cancer is the most common diagnosed female cancer. Breast cancer is also the leading cause of cancer death in females accounting for 13.7% of female cancer-related mortality globally. Variable known prognostic factors such as histological tumor type, tumor size, nodal status, grade, age, and estrogen receptor (ER) status and the proliferation marker – Ki-67 influence the type of treatment decision. The purpose of this present study is to investigate the association between Ki-67 expression with several clinicopathological variables and patients' outcome. **Materials and Methods:** This is a retrospective cohort study from September 2008 to March 2017; 165 newly diagnosed breast cancer patients were enrolled in the study. Ki67 levels were measured using immunohistochemistry and compared with clinicopathological variables. The relation of Ki67 expression with disease-free survival (DFS) and overall survival (OS) was also analyzed. **Results:** The result of this study revealed that age, tumor size, menopausal status, and human epidermal growth factor receptor 2 (HER2) status had no effect on the patients' outcome. Patients with ER-positive, progesterone receptor (PR)-positive, and HER2-negative tumors expressed a higher rate of Ki-67 (>10%) than patients with ER-negative, PR-negative, and HER2-positive tumors, respectively. However, we found that Ki-67 levels were not significantly increased statistically with ER, PR, and HER2 statuses. There was a statistically significant correlation between Ki-67 expression and with higher stages of the disease. Multivariate analysis showed that Ki-67 expression could not to be an independent prognostic factor for 5-year OS and DFS. Furthermore, p53 status was only prognostic factor for 5-year OS whereas higher stages of disease and p53 status were prognostic factors for 5-year DFS. **Conclusion:** Ki67 could not be an independent variable for prediction of breast cancer outcome.

Key words: Breast neoplasms, immunohistochemistry, Ki-67 antigen, prognosis, survival

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INTRODUCTION

Breast cancer is the most frequently diagnosed cancer worldwide. It is by far the most frequent cancer among women, with an estimated 1.67 million new cancer cases diagnosed in 2012 (25% of all cancers).[1] It is estimated that 268,670 new cases of breast cancer will be diagnosed, and 41,400 deaths will be attributed to this disease in the United States in 2018.[2] According to GLOBOCAN 2012, breast cancer ranks as the fifth cause of death from cancer overall (522,000 deaths). Incidence rates vary nearly four-fold across the world regions, with rates ranging from 27/100,000 in Middle Africa and Eastern Asia to 92 in Northern America.[3] The slight increase in breast cancer incidence from 2005 to 2014 was driven by increases of 0.3% per year among Hispanic women, 0.4% per year among non-Hispanic black women, and 1.7% per year among Asian/Pacific Islander women.[4] The incidence of breast cancer is rising in Iranian women. Age-Standardized Incidence Rate increased from 15.96/100,000 in 2003 to 33.21/100,000 in 2008.[5]

The development of new technologies and in particular, the use of complementary DNA microarrays will allow

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us now the simultaneous analysis of thousands of genes and the establishment of new, more refined breast cancer subtypes.[6] In biological molecular research, especially for breast cancer, the analysis of combining biological pathway information with gene expression data may play an important role in regulating processes involved in this disease.[7,8] For many years, tumor size, axillary lymph node status, histological characteristics of the tumor (especially histological grade of malignancy and invasion of lymphatic vessels), estrogen receptor (ER) and progesterone receptor (PR) status, human epidermal growth factor receptor 2 (HER2) expression, patient's age, and performance status were used to evaluate the prognosis and to determine the appropriate treatment strategy for breast cancer patients.[9] Recognizing of tumor proliferation is one of the important prognostic factors that determines the adjuvant treatment decision in breast cancer. Over the past few decades, proliferation markers have been evaluated as prognostic factors in breast cancer.[10]

Ki-67 labeling index (LI) and mitotic index (MI) are both proliferative indices, but their relationship is poorly defined.[11] The Ki-67 antigen is expressed in the cell cycle phases G1, S, G2, and M, but not in G0. The level of expression of the Ki-67 protein varies during the cell cycle. Rates are low in G1 and early S phase and increase to a maximum at the time of mitosis.[12] The most prevalent analysis method of Ki-67 antigen is the immunohistochemical evaluation. The rate of Ki-67 is most often measured on histological sections and is defined as the percentage of stained invasive carcinoma cells. The percentage of tumor cells expressing Ki-67 reflects the percentage of cells in the mitotic cycle within the tumor.[13]

Data on the prognostic value of Ki-67 are limited in breast cancer. Unfortunately, there is no consensus about the importance of this proliferative marker. Some researchers support the prognostic value of Ki67 in breast cancer, while others have not found the same.[14,15]

The aim of the present study was to investigate the association between Ki-67 expression with several clinicopathological variables and to assess the outcome of patients with breast cancer.

MATERIALS AND METHODS

Design and population

The study was approved by the Ethics Committee of the Tehran University of Medical Sciences. This was a retrospective cohort study. Newly diagnosed patients with breast cancer in the oncology outpatient clinic of Shariati Hospital in Tehran, Iran, between September 2008 and March 2017 were recruited for the study individuals. A total of 186 patients with breast cancer were included in this study. Exclusion criteria included metastatic disease, male gender, and those patients with incomplete data. Metastasis was detected in 21 patients, and Ki-64 data were not available in 58 cases. Therefore, inclusion criteria were met in 107 participants.

Tumor staging was performed according to tumor-node-metastasis (TNM) classification criteria. The clinicopathological factors were age, menstrual status, surgery type, lymph node involvement, tumor size, disease stage, chemotherapy, radiation therapy, and immunohistochemistry (IHC) results of ER, PR, HER2, and Ki67 status. Ki67 levels were compared with clinicopathological features. The association between Ki67 expression and disease-free survival (DFS) and overall survival (OS) was analyzed. DFS was the period after curative treatment when no disease can be detected, and OS was calculated from the time of initial diagnosis to the time of death.

Immunohistochemical staining

The samples were previously immunohistochemically stained to the manufacturer's guidelines (Ki-67 antibody; MIB-1 DAKO, dilution 1:200) and reviewed separately by second pathologists.

For IHC of Ki67, many cutoff values have been used although staining levels of 10%–20% are the most commonly used for the classification of invasive breast cancers.[16] Some researchers have described that the choice of the optimal cutoff point for IHC may depend on the clinical purpose: if Ki-67 is used to exclude patients with slowly proliferating tumors from chemotherapy protocols, a cutoff point of 10% will help avoid overtreatment. Conversely, if Ki-67 is used to identify patients sensitive to chemotherapy protocols, it is preferable to set the cutoff at 25%.[17] In this study, we preferred to use a cutoff at 10% for Ki-67 as has been found in other studies.[18-20] Specimens with <10% of stained tumor cells were defined as negative, and specimens with 10% or more of stained tumor cells were defined as a positive Ki67 expression. The patients were followed up until death or the end of the observation period (March 2017). The median follow-up duration was 49 months (range, 3–113 months).

Statistical analysis

The statistical analysis of the data was performed using the SPSS software for Windows, version 22 (SPSS Inc., Chicago, IL, USA). $P < 0.05$ was considered statistically significant. The association between clinicopathologic factors and expression of Ki-67 was determined using Chi-square/Fisher's exact tests. The results were expressed as means ± standard deviations. For assessment of

prognostic factors, univariate and multivariate analysis were performed using the Cox-proportional hazard model. The variables with $P < 0.2$ in the univariate analysis were analyzed with multivariate Cox proportional hazard model. Kaplan–Meier curves were derived to determine OS and DFS and were compared by means of the log-rank test. Median follow-up time was established with the reverse Kaplan–Meier method.

RESULTS

Characteristics of patients

A total of 165 newly diagnosed breast cancer patients were enrolled in this study. The characteristics of these patients are shown in Table 1. The average age of patients was 47.4 years (ranging from 24 to 76). It was found that most of the patients had node-negative disease (39.5%). According to TNM classification, 56.4% of the patients had T2 and 53.9% of the patients had Stage II disease. Based on histological grading results, we categorized patients into 2 groups: Group 1 (includes Grades I and II; 84.8% of the patients) and Group 2 (includes Grade III; 15.2% of the patients). Two patients (1.2%) only needed a core needle biopsy, and the rest of them (98.8%) underwent surgery (breast conservation therapy and modified radical mastectomy).

Table 1: Characteristics of breast cancer patients

Variants	Classification	Frequency (%)
Menopausal status	Premenopausal	117 (67.88)
	Postmenopausal	53 (32.12)
Lymph node involvement	No	64 (39.51)
	N1	56 (34.57)
	N2	27 (16.66)
	N3	15 (9.26)
Tumor size	T1 or <2 cm	41 (24.85)
	T2 or 2-5 cm	93 (56.36)
	T3 or 5 cm	24 (14.55)
	T4 or chest wall extension	7 (4.24)
ER	Positive	107 (64.85)
	Negative	58 (35.15)
PR	Positive	98 (59.39)
	Negative	67 (40.61)
Histological grade	Grade 1 and 2	140 (84.85)
	Grade 3	25 (15.15)
Clinical stage	Stage 1	24 (14.54)
	Stage 2	89 (53.94)
	Stage 3	52 (31.52)
HER2	Positive	40 (24.24)
P53	Positive	59 (35.76)
	Negative	106 (64.24)
Ki67	Positive	74 (69.16)
	Negative	33 (30.84)
Relapses	No relapse	132 (80.00)
	Local recurrence	9 (5.45)
	Distant recurrence	24 (14.55)

ER=Estrogen receptor; PR=Progesterone receptor; HER2=Human epidermal growth factor receptor 2

ER positivity was present in 107 patients (64.8%), and PR positivity in 98 patients (59.4%). Forty patients (24.2%) had Her2 positive disease. Ki67 was positive (>10% immunoreactive cells) in 74 patients (69.16%). The median follow-up was 5 years (61 months); nineteen patients died during this period of the time. At the end of follow-up, we used Kaplan–Meier method to estimate OS. The estimated 3- and 5-year OS were 93.31% (95% confidence interval [CI] 86.48%–96.76%) and 86.62% (95% CI 78.61%–94.63%), respectively. In addition, the estimated 3- and 5-year DFS were 87.67% (95% CI 79.71%–92.65%) and 79% (95% CI 68.19%–86.49%), respectively. Adjusted 5-year survival for disease stage was 93.75% for Stage I, 92.27% for Stage II, and 47.59% for Stage III.

Prognostic analysis

Results of prognostic analysis are shown in Table 2. We observed no statistically significant difference ($P > 0.05$) in terms of OS and DFS for age ($P = 0.32$), tumor size ($P = 0.62$), HER2 status ($P = 0.22$), menopausal status ($P = 0.78$), and tumor grade ($P = 0.05$). On the other hand, the prognostic variable with statistically significant differences for OS were ER status ($P = 0.01$), PR status ($P = 0.03$), and disease stages ($P = 0.02$). There was a significant difference between lymph node stages ($P = 0.001$) and OS in breast cancer patients, which indicated that the presence of lymph node involvement suggests a poor prognosis. However, no significant differences were observed between the positive and negative groups of Ki67 for OS and DFS. Five-year OS for Ki67-negative breast cancer was 74.22% (95% CI: 30.85%–92.72%) and 84.68% (95% CI: 71.49%–92.10%) for Ki67-positive cancers [Figures 1 and 2]. The difference between variables based on OS and DFS is summarized in Table 2. There was no significant relationship between menopausal status and Ki67. Forty-seven premenopausal patients (63.51%) and 27 menopausal patients (36.49%) had Ki67 more than 10% ($P = 0.53$).

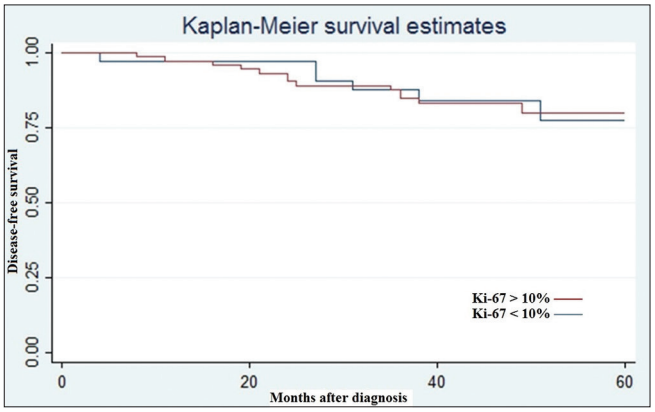

Figure 1: Kaplan–Meier survival curves for 5-year disease-free survival

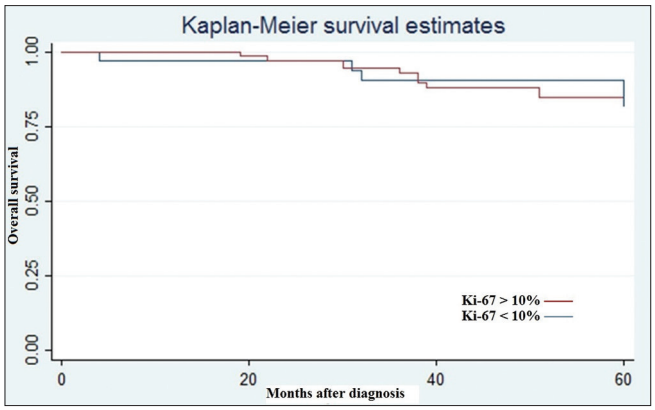

Figure 2: Kaplan–Meier survival curves for 5-year overall survival

Relationship between Ki-67 expression and the clinicopathological characteristics

The relationship between Ki-67 expression and the clinicopathological characteristics of breast cancer patients is summarized in Table 3. Patients with ER-positive and PR-positive tumors expressed a higher rate of Ki-67 (>10% immunoreactive cells) than patients with ER-negative and PR-negative tumors. However, we found that Ki67 level was not significantly increased in ER-positive and PR-positive patients (66.23% and 64.86%, respectively). Interestingly, patients with HER2-negative tumors expressed a significantly higher rate of Ki-67 (>10% immunoreactive cells) than patients with HER2-positive tumors (82.4% and 17.6%, respectively). Moreover, Ki67 level was not significantly increased in HER2-positive compared with HER2-negative patients (65% and 70.1%, respectively).

According to the pathological stage, 7 patients with Stage I (9.46%), 41 patients with Stage II (55.41%) and 26 patients with Stage III (35.14%) had a positive ki67, and significant positive correlation was demonstrated between Ki-67 and the stage of the disease ($P = 0.03$). However, no significant association was found between the involvement of lymph nodes or the grade of the disease with Ki-67 expression (0.31% and 0.19%, respectively).

Prognostic variables for mortality were analyzed by using the multivariate Cox proportional hazards model, and variables with a $P < 0.2$ in univariate analysis were used in the stepwise multivariate Cox proportional hazards model. The result of this modeling revealed that age, tumor size, menopausal status, and HER2 status had no effect on the patient's outcome; so, we used the other variable for multivariable analysis, as shown in Table 4.

In addition, we use tumor grade, tumor stages, hormone receptor, and p53 status as multivariate, and results revealed that Ki67 could not to be an independent prognostic factor for OS (heart rate [HR] 0.55, 95% CI 0.13–2.33); *P* value (0.42) and DFS (HR 1.05, 95% CI 0.30–3.62); *P* value (0.92). Multivariate analysis showed that Ki-67 expression could not to be an independent prognostic factor for 5-year OS and DFS. Furthermore, p53 status was only prognostic factor for 5-year OS whereas higher stages of disease and p53 status were prognostic factors for 5-year DFS.

Table 2: Results of prognostic analysis

Covariate	Subgroups	5-year OS (%)	*P**	5-year DFS (%)	*P**
Ki67	Positive	84.68	0.95	75.77	0.98
	Negative	74.22		81.23	
Tumor size	T1	96.30	0.098	81.79	0.04
	T2	81.68		79.08	
	T3	53.4		78.29	
	T4	-		-	
Lymph node	N0	97.50	0.007	94.71	0.001
	N1	86.86		86.86	
	N2	77.59		43.43	
	N3	20.45		45.45	
Grade	1 or 2	88.05	0.004	83.05	0.013
	3	45.93		57.08	
Stage	Stage1	93.75	0.005	86.54	0.000
	Stage2	92.77		92.77	
	Stage3	71.39		48.44	
ER	Positive	90.51	0.012	82.66	0.013
	Negative	46.20		71.33	
PR	Positive	90.07	0.064	83.44	0.004
	Negative	52.96		70.69	
HER2/neu	Positive	78.20	0.378	55.22	0.23
	Negative	88.78		83.15	
Menopause	Premenopausal	81.34	0.94	79.41	0.701
	Postmenopausal	81.40		77.11	

*Log-rank test. OS=Overall survival; DFS=Disease-free survival; ER=Estrogen receptor; PR=Progesterone receptor; HER2=Human epidermal growth factor receptor 2

DISCUSSION

Tumor markers are molecules that occur in cancer-related tissues and are useful for diagnosis, treatment, or clinical management, especially in patients with breast cancer. Ki67 is a marker of cell proliferation and has been used to stratify prognostic values in invasive breast cancer. This study was conducted to evaluate the prevalence of Ki67 as a proliferative index and to determine the prognostic and predictive value in patients with breast cancer. In addition, we tried to show the relationship between Ki67 and prognostic factors and the effect of Ki67 on the outcome of the disease. According to IHC results, 69.16% of patients had Ki67 >10%, which is considered a positive status. In line with our results, Shandiz *et al.* reported that 62.3% of patients were positive for Ki67 with a significant relation to lower age and P53 positivity.[21]

Some researchers have found that Ki67 LI not correlate with tumor size, pathologic stage, expression of ER, PR, Her-2/neu, tumor histology, breast cancer subtypes, and

Table 3: The relationship between Ki-67 expression and the clinicopathological characteristics of patients

Characteristics	Ki67		*P**
	<10%, *n* (%)	>10%, *n* (%)	
Menopausal status			
Pre	23 (32.86)	47 (67.14)	0.53
Post	10 (27.03)	27 (72.97)	
ER			
Positive	26 (33.71)	51 (66.23)	0.29
Negative	7 (23.33)	23 (76.67)	
PR			
Positive	26 (35.14)	48 (64.86)	0.15
Negative	7 (21.21)	26 (78.79)	
HER2			
Positive	7 (35)	13 (65)	0.65
Negative	26 (29.89)	61 (70.11)	
Lymph node categories			
N0	12 (30)	28 (70)	0.31
N1	16 (39.02)	25 (60.98)	
N2	2 (13.33)	13 (86.67)	
N3	3 (27.27)	8 (72.73)	
Stages			
1	9 (27.27)	7 (9.46)	0.03
2	18 (54.55)	41 (55.41)	
3	6 (18.18)	26 (35.14)	
Tumor size			
T1	13 (39.39)	14 (18.92)	0.04
T2	15 (45.45)	45 (60.81)	
T3	4 (12.12)	15 (20.27)	
T4	1 (3.03)	0 (0.00)	
Grade			
1, 2	31 (93.94)	63 (85.22)	0.19
3	2 (6.06)	11 (14.86)	

*Chi-square test. ER=Estrogen receptor; PR=Progesterone receptor; HER2=Human epidermal growth factor receptor 2

age at diagnosis.[22] Similarly, another study on 184 Iranian patients with breast cancer showed no correlation between ER and PR with p53 and Ki67.[23] These findings are not consistent with the results of the present study. In our study, we failed to find a statistically significant relationship between the level of Ki67 and menopausal status (P = 0.53), hormone receptors (P = 0.29), as well as HER 2 status (P = 0.65).

In Finland, Pietiläinen *et al*.[24] analyzed a series of 191 female breast carcinomas immunohistochemically for Ki-67 expression. In this study, Ki-67 expression was directly correlated with histological grade, the content of ER and PR, p53 accumulation, MI, S-phase fraction, and apoptotic index. However, no correlation was observed between the expression of Ki-67 and the status of lymph node, metastasis, and tumor size. In axillary lymph node-positive tumors, the expression of Ki-67 was not significantly associated with the recurrence-free survival. Multivariate survival analysis showed that tumor size, MI, and axillary lymph node status were independent prognostic factors in all cases whereas tumor size and Ki-67 expression were independent prognostic factors in axillary lymph node-negative cases. These researchers suggested that the expression of Ki-67 could be an important prognostic factor in breast cancer.[24]

Our results revealed that Ki67 was associated with stage of breast cancer (P = 0.03), indicating that a high levels of Ki67 are found in more invasive tumors. In line with this result, results of a large population-based cohort of a cancer registry reported that Ki67 expression was associated with common histopathological parameters but was an additional independent prognostic parameter for DFS and OS in patients with breast cancer. In this study, the strongest correlation was found between grading and Ki67. In addition, they showed that higher tumor stages and node status were associated with higher Ki-67 quartiles, suggesting that the more aggressive tumor had a higher percentage of cells positively stained for Ki67.[25] In another study, Abubakar *et al*.[26] reviewed 8088 breast cancer patients from 10 study groups and showed that patients in the highest quartile of Ki67 (>12% positive Ki67 cells; that is close to our limit) had a worse 10-year breast cancer-specific survival than patients in the lower three quartiles. This relationship was statistically significant for ER-positive patients but not for ER-negative patients. Among the ER-positive cancers, Ki67 was accompanied by a worse prognosis in both node-negative and node-positive tumors. In 2011, Soliman and Yussif[27] performed a study to determine the clinical significance of Ki-67 index in different molecular subtypes of 107 patients with breast cancer. They concluded that patients with Ki67 <15% experience better OS than those with higher levels of Ki67. In addition, patients with Ki-67 higher than 15% were significantly correlated with adverse prognostic factors, high mitotic count, high tumor grade, ER−/PR−, higher incidence of metastasis, and recurrence than those with Ki-67 <15%.

Our study was unable to find out the effect of Ki67 in OS and DFS (P = 0.42 and P = 0.92, respectively). In addition, similarly to other literature, which indicates a significant impact of tumor grade, disease stage, and lymph node involvement in OS and DFS,[28] the present study showed that the same variable was associated with a negative impact on OS and DFS. Unlike the studies previously mentioned, in the present study, we had not found any prognostic significance for ER and PR receptors regarding OS and DFS. In our study, the grade of tumor has not been statistically significant value for DFS and OS. These findings are inconsistent with the results of some studies.[29]

The findings in this study are subject to several limitations. First, this study was a single center with a limited sample size, and these results may not be generalizable to other centers. Large-scale population studies are necessary to

Table 4: Univariate and multivariate Cox proportional hazards model analysis

Covariate	OS				DFS			
	Univariate		Multivariate		Univariate		Multivariate	
	HR (CI 95%)	*P*	HR (CI 95%)	*P*	HR (CI 95%)	*P*	HR (CI 95%)	*P*
Age	0.97 (0.92-1.02)	0.33	-		0.99 (0.95-1.03)	0.76	-	
Size	1.06 (0.83-1.36)	0.61	-		1.03 (0.83-1.28)	0.74	-	
Grade								
I/II	Reference		-		-		-	
III	3.01 (0.92-9.81)	0.06*	3.38 (0.88-12.9)	0.07	2.40 (0.86-6.67)	0.09*	2.29 (0.71-7.39)	0.16
Stage								
I	Reference	-	-		Reference		Reference	
II	1.05 (0.11-9.47)	0.96	0.83 (0.07-9.45)	0.88	0.49 (0.09-2.72)	0.42	0.42 (0.70-2.53)	0.34
III	4.27 (0.53-34.2)	0.17*	5.86 (0.52-65.9)	0.15	3.62 (0.81-16.0)	0.09*	6.14 (1.06-35.3)	0.04**
ER PR								
Negative	Reference		-		Reference		Reference	
Positive	0.26 (0.08-0.80)	0.02*	0.35 (0.10-1.23)	0.10	0.44 (0.17-1.11)	0.08*	0.59 (0.20-1.76)	0.35
P53								
Negative	Reference		-		Reference		Reference	
Positive	9.5 (2.12-43.2)	0.003*	13.47 (2.7-66.1)	0.001**	3.89 (1.47-10.2)	0.006*	5.47 (1.86-16.0)	0.002**
Ki67								
Negative	Reference		-		Reference		Reference	
Positive	1.03 (0.31-3.36)	0.95	0.55 (0.13-2.33)	0.42	1.01 (0.38-2.66)	0.98	1.05 (0.30-3.62)	0.92
Menopausal status								
Premenopausal	Reference		-		-		-	
Menopause	1.17 (0.38-3.59)	0.78	-		1.419 (0.56-3.50)	0.45	-	
Her2								
Negative	Reference	-	-	-	Reference	-	-	-
Positive	2.16 (0.66-7.05)	0.21			2.11 (0.80-5.59)	0.13*		

**P*<0.20 in the univariate analysis was included in the multivariate analysis, ***P*<0.05 in the multivariate analysis was considered statistically significant. Ki-67 was the main independent variable and was included in the subsequent multivariate analysis. OS=Overall survival; DFS=Disease-free survival, ER=Estrogen receptor; PR=Progesterone receptor; HER2=Human epidermal growth factor receptor 2; CI=Confidence interval; HR=Heart rate

confirm these observations. Second, this study did not assess to precise evaluation of the relationship between Ki67 and the relapse risk.

CONCLUSION

According to the results of the present study, Ki67 could not be used as an independent prognostic factor for invasive breast cancers. It was also concluded that there is no significant relationship between Ki67 and some prognostic factors such as hormonal receptors and HER2. In addition, no significant difference was observed between Ki67 and 3- and 5-year DFS with 5-year OS.

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Conflicts of interest

There are no conflicts of interest.

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