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Original Article

# Comparison of Cerb B2 expression and Ki-67 index with modified scarff-bloom-richardson grading system in invasive ductal carcinoma

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**Abstract**

**Aim:** Breast cancer is the most frequent cancer among women. Cerb B2 is an oncogene that encodes a transmembrane glycoprotein known as HER-2 protein or receptor. Ki-67 is nuclear protein associated with cellular proliferation. In this study, we investigated the relationships between the histopathologic parameters in the "Modified Scarff-Bloom-Richardson" microscopic grading and expression of Cerb B2 and Ki-67 index gene in cases with invasive ductal carcinoma. Breast cancer is the most common type of cancer in women, but also the second most common cause of death after lung cancer.

**Material and Methods:** In this study examined 45 patients who were diagnosed with invasive ductal carcinoma between January 2017 and January 2019 in Sivas Cumhuriyet University, department of surgical oncology. Cases, Cerb B2 expression and Ki-67 index were retrospectively screened from pathology records

**Results:** Increased expression of Cerb B2 was statistically significant related with increased tubule formation, pleomorfism, mitosis rate, and histological grade (p=0.006, p=0.027, p<0.001, p=0.001, respectively). Increased Ki-67 index was statistically significant related with increased tubule formation, pleomorphism, mitosis rate, and histological grade (p=0.031, p<0.001, p=0.048, p=0.010, respectively).

**Conclusion:** As a result, it was observed that there was a positive correlation between parameters included in Modified Scarff-Bloom-Richardson grading system with Cerb B2 expression and Ki-67 index. Cerb B2 and Ki-67 index are important prognostic biomarkers in human breast cancer.

**Keywords:** Cerb B2, invasive ductal carcinoma of the breast, Ki-67

## INTRODUCTION

Breast cancer is the most common type of cancer in women, but also the second most common cause of death after lung cancer (1). Despite the development of early diagnosis methods and advances in treatment, breast cancer is still an important cause of morbidity and mortality. Invasive ductal carcinoma constitutes 75-80% of breast carcinomas. It is the most important group of malignant breast tumors that cannot be classified in any of the special types (2).

Nowadays, it is the scoring system modified by Elston and Ellis which was used by Bloom and Richardson in 1957 in grading of invasive ductal carcinoma (3). In this grading system, the nuclear characteristics of the tumor cells, the ratio of the tubular structures they form and the number of mitosis are graded between 1-3 and the grade is determined according to the total score obtained (3,4). Grade 1; well differentiated (3-5 points), grade 2; moderately differentiated (6-7 points) grade 3; poorly differentiated (8-9 points). Bloom and Richardson stated that the histological grade reflects the degree of potential malignancy. Since histological grade is also considered as a prognostic factor, the relationship between grade and many factors has been investigated (5).

The Cerb B2 gene; It is defined as p185 localized on chromosome 17 in an area where genetic instability is common in breast cancer. The Cerb B2 gene; The 1255 amino acid is oncogene that encodes a transmembrane glycoprotein, known as HER-2 (Cerb B2) protein or receptor, which is one of the 4 members of the EGF (epidermal growth factor) receptor family (6). Human epidermal growth factor receptor-2 (HER-2) expressions are used as prognostic and predictive factors. Increased expression and amplification of Cerb B2 has been reported in breast

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carcinomas ranging from 20-40%. In breast cancer, 92% of cases that show Cerb B2 protein overexpression occur as a result of HER-2 gene amplification (7). Preclinical studies have shown that Cerb B2 anomalies result in a more malignant phenotype (8). Consistent with the preclinical results, numerous clinical studies have shown that breast cancers with abnormal Cerb B2 have a worse prognosis (9). Ki-67 that Gerdes et al. were found at theirs's investigate at Hodgkin's lymphoma patient in 1980, is a nuclear protein related to cellular proliferation. Ki-67 is generally evaluated immunohistochemically. Ki-67 is not expressed in G0 from cell cycle stages but is found in other S, G1, G2 and M phases. Disease-free survival and overall survival have been shown to be worse in patients with high Ki-67 expression. As a result, it is emphasized that cytosolic samples should be examined for Ki-67 in breast cancer patients. There is a good correlation between Ki-67 nuclear immunoreactivity showing cell proliferation and the number of mitosis in tumor tissue. With this antibody it is possible to detect the growth fraction in a given cell population. The proliferation index roughly correlates with the tumor stage and also has an independent prognostic value in many cancers (10).

Because of the variable tumor behavior in patients receiving the same treatment, the need for new prognostic factors is increasing in order to predict survival, relapse prevention and to use treatment regimens more effectively (11). In our study, 45 patients diagnosed as invasive ductal carcinoma between January 2017 and January 2019 were included. The pathology reports of the patients with breast cancer were reviewed retrospectively. The aim of this study was to understand the prognostic significance of Cerb B2 and Ki-67 in invasive ductal carcinoma. This study was evaluated whether there is a significant relationship between tubular formation in the modified Scarff-Bloom-Richardson grading system, nuclear pleomorphism, mitosis rates and the sum of these grades and Cerb B2 staining patterns and Ki-67 indexes.

## MATERIAL and METHODS

Forty-five patients who underwent surgery at Sivas Cumhuriyet University Surgical Oncology Department between January 2017 and January 2019 and diagnosed as invasive ductal carcinoma after examination in the pathology department were included in the study. The pathology records of the cases were retrospectively reviewed.

Tumors were scored using a modified Scarff-Bloom-Richardson grading system. The tubular formation in the tumor by mitosis and nuclear pleomorphism was determined and the score was made as 1-3. Accordingly, when evaluating tubule formation, if more than 75% of the tumor shows prominent lumen tubules, 1 point; if there is a tubule structure between 10-75% with solid areas, 2 points; if tubular structure below 10% or none, 3 points was evaluated. Nuclear pleomorphism; 1 point if the tumor cells have uniform, regular and small nuclei; 2 points if nuclei differ moderately and nuclei can be selected in some places, 3 points if they vary greatly and contain one or more prominent nucleoli was evaluated.

The number of mitosis was evaluated as 1 point in 0-9, 2 points in 10-19, and 3 points in 20 and above. According to these parameters, the total score was evaluated as 3-5 grade 1, 6-7 grade 2, 8-9 score grade 3.

In the pathology of the biopsy taken from the related tissue that the patients were diagnosed with breast cancer; Cerb B2 immunohistochemical stains were grouped as 0, 1+, 2+, 3+ according to the dye uptake of the tissues. Percentage of Ki-67 proliferation index (%) was recorded.

### Statistical analysis

SPSS 15.0 Windows program was used for statistical analysis. Descriptive statistics were given as the number and percentage for categorical variables mean, standard deviation, minimum, and maximum for numerical variables. Differences between categorical variables in independent groups were tested by Chi-square analysis. Monte-Carlo simulation was applied when the conditions were not met. The relationship between ordinal variables and numerical variables was analyzed by Spearman Correlation analysis. $p < 0.05$ was considered significant.

## RESULTS

The ages of the patients included in the study were between 25-76 years and the mean age was 54.2 ± 9.5 years. 45 of the cases were female. 29 right and 26 left breast cancer patients were performed Lumpectomy in 21 cases and modified radical mastectomy in 24 cases. Although the diameter of the tumors varies between 0.8-9 cm; mean 2,9 ± 1,5 cm and more than one focal placement was observed in 4 cases.

Tubular formation was evaluated as 1 point in 14 (31.1%) cases, 2 points in 24 (53.3%) cases and 3 points in 7 (15.5%) cases. Nuclear pleomorphism was evaluated as 1 point in 8 (17.7%) cases, 2 points in 17 (37.7%) cases and 3 points in 20 (44.4%) cases. Mitosis was evaluated as 1 point in 5 (11.1%) cases, 2 points in 25 (55.5%) cases and 3 in 15 (33.3%) cases. The histologic grade was 1 in 24 (53.3%) cases, 2 in 15 (33.3%) cases and 3 in 6 (13.3%) cases.

As a result of Cerb B2 staining and Ki-67 indixes, that had applied in cases; There was no staining in 12 (26.6%) cases for Cerb B2; was seen staining 0.0 (0.00%) cases in 1+, 15 (33.3%) cases in 2+, and 18 (40%) cases 3+. In the evaluation of Ki-67 index; Group 1 was made below 15%, group 2 was between 15-30% and group 3 was above 30%. Accordingly, 19 (42.2%) cases were in the first group, 11 (24.4%) were in the second group, 15 (33.3%) were in the third group. The level of staining of Cerb B2; There was a statistically significant relationship between tubule formation, nuclear pleomorphism, mitosis and histological grade levels ($p = 0.006$, $p = 0.027$, $p < 0.001$, $p = 0.001$, respectively). The increase in tubule formation, mitosis and Cerb B2 levels in grade 3 was remarkable. This relationship is given in Table 1 and Figure 1.

**Table 1. Cerb B2 relationship between tubule formation, nuclear pleomorphism, mitosis, and histological grade levels**

| | | Cerb B2 | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | 0 | | 1 | | 2 | | 3 | | |
| | | n | % | n | % | n | % | n | % | p |
| Tubule formation | 1 | 5 | 35.7 | 0 | 0.0 | 7 | 50.0 | 2 | 14.3 | |
| | 2 | 7 | 29.2 | 0 | 0.0 | 8 | 33.3 | 9 | 37.5 | 0.006 |
| | 3 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 7 | 100.0 | |
| Nuclear pleomorphism | 1 | 4 | 50.0 | 0 | 0.0 | 4 | 50.0 | 0 | 0.0 | |
| | 2 | 1 | 5.9 | 0 | 0.0 | 7 | 41.2 | 9 | 52.9 | 0.027 |
| | 3 | 7 | 35.0 | 0 | 0.0 | 4 | 20.0 | 9 | 45.0 | |
| Mitosis | 1 | 5 | 100.0 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | |
| | 2 | 5 | 20.0 | 0 | 0.0 | 13 | 52.0 | 7 | 28.0 | <0.001 |
| | 3 | 2 | 13.0 | 0 | 0.0 | 2 | 13.3 | 11 | 73.3 | |
| Histological grade | 1 | 11 | 45.8 | 0 | 0.0 | 8 | 33.3 | 5 | 20.8 | |
| | 2 | 1 | 6.7 | 0 | 0.0 | 7 | 46.7 | 7 | 46.7 | 0.001 |
| | 3 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 6 | 100.0 | |

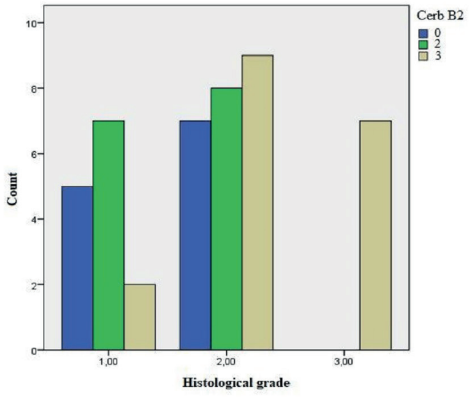


**Figure 1.** Cerb B2 relationship between histological grades

There was a statistically significant relationship between the level of Ki-67 index and tubule formation, nuclear pleomorphism, mitosis and histological grade levels (p = 0.031, p <0.001, p = 0.048, p = 0.010, respectively). Nuclear pleomorphism, mitosis and Ki-67 index level increase in grade 3 was remarkable. This relationship is given in Table 2 and Figure 2.

Cerb B2 was the survival of 100% in the group with 0, the survival of 93.3% in the group with 2 +, and the survival of 66.6% in the group with 3+ (Figure 3). There was a significant difference according to the Breslow (Generalized Wilcoxon) analysis (p = 0.006). Cerb B2, the patients of 3+ the group are significantly 6 times more likely to die than the patients of 2 + the group (OR = 13.96, 95% Confidence interval (1.92 - 21.63) (Figure 4).

**Table 2. Ki-67 relationship between tubule formation, nuclear pleomorphism, mitosis, and histological grade level**

| | | Ki-67 | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | 1 | | 2 | | 3 | | |
| | | n | % | n | % | n | % | p |
| Tubule formation | 1 | 7 | 87.5 | 1 | 12.5 | 0 | 0.0 | |
| | 2 | 6 | 35.3 | 6 | 35.3 | 5 | 29.4 | 0.031 |
| | 3 | 6 | 30.0 | 4 | 20.0 | 10 | 50.0 | |
| Nuclear pleomorphism | 1 | 5 | 100.0 | 0 | 0.0 | 0 | 0.0 | |
| | 2 | 12 | 48.0 | 10 | 40.0 | 3 | 12.0 | <0.001 |
| | 3 | 2 | 13.3 | 1 | 6.7 | 12 | 80.0 | |
| Mitosis | 1 | 13 | 54.2 | 6 | 25.0 | 5 | 20.8 | |
| | 2 | 5 | 33.3 | 5 | 33.3 | 5 | 33.3 | 0.048 |
| | 3 | 1 | 16.7 | 0 | 0.0 | 5 | 83.3 | |
| Histological grade | 1 | 10 | 71.4 | 4 | 28.6 | 0 | 0.0 | |
| | 2 | 8 | 33.3 | 6 | 25.0 | 10 | 41.7 | 0.010 |
| | 3 | 1 | 14.3 | 1 | 14.3 | 5 | 71.4 | |

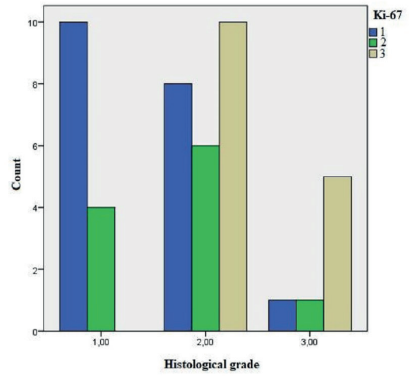


**Figure 2.** Ki-67 relationship between histological grades

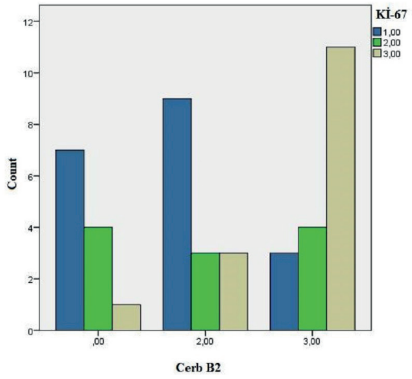


**Figure 3.** Ki-67 relationship between Cerb B2

**Table 3. Relationship between Cerb B2 and Ki-67**

| | | Ki-67 | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | 1 | | 2 | | 3 | | |
| | | n | % | n | % | n | % | p |
| Tubule formation | 0 | 7 | 58.3 | 4 | 33.3 | 1 | 8.3 | 0.015 |
| | 1 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | |
| | 2 | 9 | 60.0 | 3 | 20.0 | 3 | 20.0 | |
| | 3 | 3 | 16.7 | 4 | 22.2 | 11 | 61.1 | |

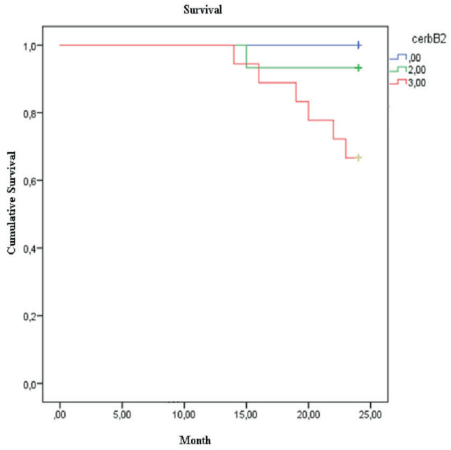


**Figure 4.** Kaplan Meier survival curve showing the relationship between Cerb B2 and survival

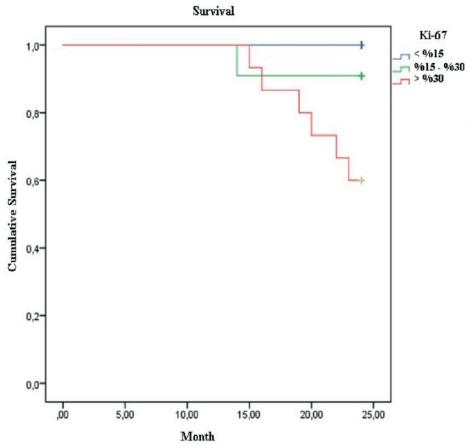


**Figure 5**. Kaplan Meier survival curve showing the relationship between Ki-67 and survival

The survival was 100% for those who expressed Ki-67 at 0-15 % density, the survival was 90.9 % for those who expressed at 15-30 % density, and the survival was 60.0 % for those who expressed > 30% (Figure 5). There was a significant difference according to the Breslow (Generalized Wilcoxon) analysis (p = 0.006).

According to the analysis result, Ki-67 patients in the group above 30% have a significantly five times higher risk of death than those in the 15-30% range. (OR = 4.99, 95% Confidence interval = 1.34 -18.60) (Table 3).

## DISCUSSION

The most common cancer in women is breast cancer. The etiology of breast cancer is multifactorial and many factors such as genetic disposition, hormonal status, diet, reproductive factors, alcohol, body weight and physical activity play a role. The most important prognostic factors in terms of treatment and survival are axillary lymph node metastasis, tumor stage, tumor size, degree of differentiation, lymphatic invasion, hormonal status, and distant metastasis.

The most common breast tumor is invasive ductal carcinoma (12).

Cerb B2 encodes the tyrosine kinase receptor. It is takes charge in cell growth and differentiation. Mitogenic effect increases with hyperactivation. As a result of increased signal transduction, cell cycle control mechanisms are affected. Cerb B2 mutations increase mitogenic effect via the cyclin D1 / CDK complex, one of the cell cycle control mechanisms. Apart from the oncogenetic effect, it plays a role in the development of resistance to chemotherapy (13). Increased expression of HER2 / neu, a well-defined proto-oncogen, is known to be associated with aggressive behavior and poor prognosis in recent studies. Her2 positive breast tumors differ clinically from negative tumors. It occurs at a younger age and tends to be more hormone receptor negative. Axillary lymph node metastasis is more common in Her2 positive breast tumors and most of them are high grade It has been reported that there is a strong correlation between HER2 / neu overexpression and aggressive biological behavior and poorly differentiated ductal carcinoma in situ (7).

In our study, 11 (91.6%) of the Cerb B2 negative cases were grade 1, 1 (8.3%) grade 2, and 0 (0.0%) grade 3 There were no +1 positive cases according to Cerb B2 staining level. According to the Cerb B2 staining level 2 positive cases; 8 (53.3%) were grade 1, 7 (46.6%) were grade 2, 0 (0.0%) were grade 3. According to the Cerb B2 staining level 3 positive cases; 5 (27.7%) were grade 1, 7 (38.8%) were grade 2, 6 (33.3%) were grade 3. Cerb B2 positivity significantly increased as histologic grade increased (p = 0.001).

Tubular formation was evaluated as 1 point in 14 (31.1%) cases, 2 points in 24 (53.3%) cases and 3 points in 7 (15.5%) cases. There was a statistically significant relationship between staining levels of Cerb B2 and tubule formation levels (p = 0.006). The increase in Cerb B2 staining level was remarkable in the group with tubule formation scored as 3 As the degree of tubule formation increased, there was a significant increase in Cerb B2 staining level (p = 0.006).

Nuclear pleomorphism was evaluated as 1 point in 8 cases (17.7%), 2 points in 17 cases (37.7%) and 3 points in 20 cases (44.4%). There was a statistically significant relationship between Cerb B2 staining level and nuclear pleomorphism (p = 0.027).

In our study, 5 cases (11.1%) were evaluated as 1 point, 25 cases (55.5%) as 2 points and 15 cases (44.4%) as 3 points according to mitosis levels. There was a statistically significant relationship between Cerb B2 staining level and mitosis levels (p <0.001). The increase in Cerb B2 level was remarkable in the group whose mitosis level was scored as 3.

Cerb B2, which is accepted as an indicator of tumor aggression, shows a close similarity with other proliferation markers in the studies performed (14).

The first study showing the relationship between Cerb B2 gene amplification and prognosis in breast carcinomas was made by Slamon et al. According to this study, Cerb B2 oncogen amplification detected by southern-blot technique showed that it was not correlated with tumor size, estrogen and progesterone receptor status and age but had independent prognostic importance in patients with lymph node metastasis (15). In the study of Borg et al. In 539 invasive primary breast carcinomas, a correlation between large tumor size, positive lymph node status, advanced stage and absence of steroid receptors and increased expression of Cerb B2 has been shown (16).

Marx et al. Found no significant relationship between lymph node status and tumor size and Cerb B2 expression, and found that Cerb B2 expression was significantly higher in high grade tumors (16). In our study, Cerb B2 expression was associated with tumor size (mean size 2.9 ± 1.5 cm) and high grade.

The relation of Cerb B2 with the parameters forming the grade formation in breast cancers was investigated and it was found that the connection of Cerb B2 with nuclear grade was stronger than that of tubule formation (17). In our study, the relationship between Cerb B2 expression and mitosis number was found to be stronger than its relationship with nuclear grade and tubule formation.

HER2 height is more common in breast cancers with high histological grade, estrogen and progesterone negativity and high proliferation index (15). Overall, there is a correlation between survival and HER2. Although HER2 overexpression rate has been reported in the literature between 15-25%, it was found to be higher in younger patients (18).

The nuclear related antigen Ki-67 protein is a nuclear protein expressed during cellular proliferation. Overexpression of Ki-67 corresponds to the high proliferation rate of tumor cells and is considered as an independent factor for poor prognosis in breast cancer patients (19). Ki-67, a gene on chromosome 10, has been shown to detect a nuclear protein in the nuclear matrix of proliferating cells and its function is associated with mitosis. In particular, it is closely associated with tumor cell proliferation (8).

There are many studies in the literature regarding the prognostic significance of Ki-67 index in tumor tissue. In these studies, Ki-67 threshold value was taken as diverse values in different studies. According to our study; 19 cases (42.2%) in 1 group with Ki-67 index less than 15%, 11 cases (24.4%) in 2 groups between 15-30% and 15 cases in 3 groups with more than 30% 33.3%).10 (52.6%) of the patients with Ki-67 index in the first group were grade 1,8 (42.1%) were grade 2, 1 (5.2%) was grade 3. 4 (36,3%) of the patients with Ki-67 index in the second group were grade 1,6 (54,5%) were grade 2, 1 (9,0%) was grade 3 (9,0%). 0 (0,0%) of the patients with Ki-67 index in the third group were grade 1, 10 (66,6%) were grade 2, 5 (33,3%) were grade 3. There was a statistically

significant relationship between the level of Ki-67 index and histological grade level (p = 0.001). There was a statistically significant correlation between Ki-67 index and tubule formation, nuclear pleomorphism and mitosis levels (p = 0.031, p <0.001, p = 0.048, respectively). Raise of Nuclear pleomorphism, mitosis, and Ki-67 index level at grade 3 were remarkable. In the largest meta-analysis study were taken 64,196 patients and was evaluated the prognostic significance of different thresholds of Ki-67 in breast cancer patients. No clear results could be obtained regarding the prognostic significance of Ki-67 percentage. However, a threshold value of 25% was found to be more significant in terms of survival (20). In a study of 1475 cases in which Ki-67 score was evaluated, the threshold value was found to be ≤15% (21).

This meta-analysis confirmed that high Ki-67 levels were associated with worse prognosis (22). They showed that Ki-67 positivity provides a higher risk of relapse and poorer survival in both LN positive and negative groups.

212 patients in whom Ki-67, p53 and estrogen receptors were evaluated had lower survival in patients with high Ki-67 expression (> 10%) with pTINOM0 breast carcinoma. however, this was not significant according to failure (23).

In a study of 3,658 non-metastatic patients; Ki-67 was determined as a prognostic parameter independent of clinical and histopathological factors. The five-year survival rate was 86.7% in patients with Ki-67 <15% and 75.8% in patients with> 45%. Low pT stages were associated with low expression of Ki-67. Ki-67 was very low in grade 1 tumors and very high in high grade tumors. Cerb B2 positivity was found in tumors with high Ki-67 (24). Similarly, in our study, Ki-67 index increased with increasing grade. In the present study, Ki-67 levels were mostly 20% or more in the Cerb B2 positive groups.

Tamaki K. et al. found a significant positive correlation between Ki-67, ER / Cerb B2, and grade in 480 patients at Japan, similar to our study (25).

In 37 patients with estrogen receptor negative breast cancer without lymph node metastasis, high Ki-67 was found to be associated with Cerb B2 positivity (Ki > 20%). It has been observed that Ki-67 is a reliable and independent prognostic indicator in such patients (26).

Pérez-López M. et al. Luminal B, lymph node negative in 888 patients with breast cancer Ki-67 's were evaluated. It was found that the biological parameter related to poor survival was Ki-67, progesterone receptor and Cerb B2 had no prognostic significance (27).

In the study performed by Hao S et al., 571 patients who evaluated the prognostic effect of Ki-67 index in triple negative patients were included in the study; if Ki-67 was above 35%, had a poor prognosis in triple negative patients diagnosed under age 50 (28). In our study, Ki-67 index of triple negative patients was over 30%.

Weisner et al.2523 patients were in the study, Ki-67 index was a prognostic factor independent of grade, Her2 status and histologic tumor type. Ki-67 index greater than 20% was found to be associated with all prognostic factors (29).

## CONCLUSION

As a result; the prognosis of patients with similar characteristics varies during the course of breast cancer treatment. This raises the need for new prognostic factors. In our study, similar results were obtained with the literature. Although Cerb B2 and Ki-67 were thought to be a prognostic factor for the prognosis of breast tumor, the fact that the study was performed retrospectively, whether the fixation was performed adequately during macroscopy and the nature of the breast tissue were limitations for us.

*Competing interests: The authors declare that they have no competing interest.*

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*Ethical approval: There is no research involving direct intervention on humans or experimental animals, no treatment methods or tools have been used, only retrospectively screened data.*

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