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# EVALUATION OF KI-67 AS INDEPENDENT RISK FACTOR AND ITS ROLE IN THE INCIDENCE OF LOCAL RECURRENCE/DISTANT METASTASIS IN LUMINAL A AND LUMINAL B (HER2 NEGATIVE) BREAST CANCER: A RETROSPECTIVE ANALYSIS FROM A SINGLE CANCER CENTER

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**ABSTRACT – *Objective:*** We aimed to assess the relationship between the Ki-67 index and the risk of recurrences and survival in patients with breast cancer (BC) that had positive estrogen receptor (ER), positive progesterone receptor (PR), and negative human epidermal growth factor receptor (HER2).

***Patients and Methods:*** A total of 108 patients who visited the Clinical Oncology Department at Assuit University Hospital between 2015 and 2018 were involved in the study. The level of Ki-67 was measured and patients were divided into low Ki-67 (n=62) and high Ki-67 (n=46) groups using 14% as the cut-off value. The Cox-regression hazard model was used for both Univariate and Multivariate analyses. Kaplan-Meier survival curves were used for the survival analysis.

***Results:*** Age, menopausal status, performance status (PS), pathological type, tumor stage (T), nodal stage (N), grade (G), and TNM stage were all analysed in relation to the Ki-67 index; the only statistically significant variable was the T stage ($p$=0.043). Patients with high Ki-67 level had a greater mortality rate than those with low levels ($p$=0.004). In comparison to low index groups, the mean disease free survival (DFS) and overall survival (OS) were lower in the high index groups (DFS: 48.41± 4.19 months *vs.* 64.53± 2.48 months and OS: 54.74± 3.59 months *vs.* 66.54± 1.99 months with $p$=0.001 and 0.002, respectively). When compared to the low index group, the high Ki-67 group had a significantly higher incidence of local recurrence (LR) and metastasis ($p$=0.001).

***Conclusions:*** In patients with positive ER/PR and HER2, negative HER2 BC, the level of Ki-67 strongly inversely correlates with LR/metastasis, DFS, and OS.

**KEYWORDS:** Ki-67, LR/metastasis, Survival, ER/PR +ve, HER2 –ve, BC.

## INTRODUCTION

The experts are attempting to understand the biological behavior of the disease as the prevalence of cancer rises each year. The identification of cancer biomarkers (CBs) was one of the most significant advancements. According to the papyrus, the ancient Egyptians were the first to employ tumor markers to distinguish between breast tumors and mastitis roughly 2000 years ago [1]. The first CB discovered in multiple myeloma patients was the Bence-Jones protein in 1847 [2]. Since then, a large number of tumor markers have been identified. Both predictive and diagnostic tumor markers are available [3]. Breast cancer (BC) is the most frequent cancer in women worldwide. A total of 43,250 deaths and 287,850 new cases were predicted to occur in the United States in 2022 [4]. However, BC is a diverse disease with a wide range of morphological and molecular subtypes, so its presentation, behavior, and outcome vary. As a result of advancements in adjuvant treatment, the mortality rate was reduced by nearly 50%. Numerous indicators have been investigated over the past few decades to categorize BC into subtypes, making it easier to understand its biological behavior to enhance outcomes, and forecast how it will react to newly emerging targeted therapies. There are several independent but interrelated prognostic factors predictive of recurrence and survival including; axillary nodal status, the human epidermal growth factor receptor (HER2), the progesterone receptor (PR), the estrogen receptor (ER), Ki-67 proliferation index, DNA ploidy, and oncogene amplification. S-phase fraction can also be used to define the high-risk patient [5-9]. Expression of the Ki67 protein is associated with the proliferative activity of intrinsic cell populations in malignant tumors, allowing it to be used as a marker of tumor aggressiveness [10,11]. Ki-67 or antigen Ki-67, often known as the Marker of Proliferation is a human protein that is encoded by the MKi-67 gene. Ki-67 is expressed throughout the cell cycle, with the exception of the G0 phase, peaking in the M phase and declining in the G1 and S phases. As a result, it is frequently utilized to demonstrate the proliferation of cancer cells. Immunohistochemistry (IHC) allows for the visualization of this expression, which can then be photographed, quantified, and represented as a percentage of the total number of cells [12-16]. Due to the proliferative activity of cancer cells, the Ki-67 proliferation index is frequently utilized as a predictive biomarker in numerous malignancies, including BC, and used to assess the response to systemic treatment [17-27]. The correlation between the Ki-67 index and other BC prognostic variables such as age, grade (G), lymph-vascular invasion (LVI), nodal stage (N), tumor stage (T), ER, PR, and HER2 was examined in a number of studies [28-33]. Our study's objective was to determine whether there was a relationship between the Ki-67 index in early BC with ER/PR positivity and HER2 negativity and the likelihood of local recurrence (LR) or distant metastasis as well as disease survival when compared to other prognostic variables.

## PATIENTS AND METHODS

### Patients

This retrospective study was carried out from January 2015 to December 2018. A total of 108 out of 517 female patients who were diagnosed with BC and who had early-stage disease (317 cases) were eligible for the study.

Inclusion criteria were: a) patients between the ages of 18 and 70 at the time of diagnosis; b) have pathological confirmation of invasive breast cancer (IBC); c) be in the early stages of the disease according to the 8th Edition of the American Joint Committee on Cancer (AJCC); d) have IHC results that were positive for ER, PR, and negative for HER2; e) have undergone either a modified radical mastectomy (MRM) or conservative surgery (CS); f) and have full data from a three-year follow-up.

### Ethical Approval

The study was approved by the Ethical Committee of Faculty of Medicine, Assiut University with IRB local approval number: 17300889.

### Study methodology

Tumor slides that had been formalin-fixed, paraffin wax-embedded, and stained with hematoxylin and eosin were used to assess the post-operative tumor specimen. It identified the tumor's G, size, and

type, as well as the number of lymph nodes that were positive. The AJCC TNM staging system was used to evaluate the pathological staging. IHC was carried out to evaluate the ER, RP, HER2 status, and Ki-67 index. According to American Society of Clinical Oncology/College of American Pathologists (ASCO/CAP) guidelines, cancer cells were considered ER/PR positive if ≥ 1% of the cells had stained nuclei, depending on the degree of staining and the percentage of cells exhibiting nuclear staining [34]. The quantity and intensity of cell membrane staining determine the HER2 status; if fewer than 10% of cancer cells were stained, HER2 was regarded as negative [35]. Evaluation of the Ki-67 index is based on the percentage of stained cells, not the intensity of stained cells. We divide our patients into two groups based on their Ki-67 index readings, low Ki-67 index < 14% and high Ki-67 index >14%, as there is no established cut-off point. This cut-off point was recommended at the St. Gallen conference in 2011 and was frequently adopted by multiple prior studies[36].

### Definitions

The last censorship was carried out and the study was ended in December 2021. A clinical and radiological examination was performed as a follow-up on every patient. Examining the relationships between the Ki-67 index and the other prognostic parameters was the main goal of the study. The secondary endpoints were overall survival (OS), which was calculated from the time of diagnosis to the time of death from any cause or the last follow-up, and disease-free survival (DFS), which was calculated from the time of diagnosis to the time of recurrence, metastasis or death.

### Statistical Analysis

Numerical data are reported with mean and standard deviation (SD), and descriptive statistics were used to analyze patient and tumor features as numbers and percentages. The Statistical Package for Social Sciences (SPSS) software version 22.0 (IBM, Armonk, NY, USA) was used to conduct the statistical analysis. Using Pearson's Chi-square test and Fisher's exact test, the relationship between Ki-67 groups and other clinicopathologic characteristics was assessed. The Kaplan Meier test was used to assess survival analysis results. Statistical significance was defined as a *p*-value less than 0.05.

## RESULTS

The hospitalization records for patients with early BC diagnosis from January 2015 to December 2018 were used in this retrospective study. As seen in Figure 1, there were 108 patients who met the inclusion criteria. The mean age of our enrolled patients was 50.11 ± 10.79 years. Age, menopausal status, performance status (PS), type of pathology, N stage, G of the tumor, and stage of disease were not significantly different between low (n=62) and high (n=46) Ki-67 index groups; however, the only significant variable was T stage with a *p*-value of 0.043, as shown in Table 1.

At the time of the study's cutoff in December 2021, approximately 74.0% of the patients were still living, whereas 28 (26.0%) were either missed or died. In the low Ki-67 index group, 53 patients (88.5%) were alive, compared to patients in the high Ki-67 index group, where only 27 patients (58.7%) were alive, with a significant difference ($p$=0.004). The mortality rate in the low Ki-67 group was also lower than that in the high Ki-67 group. In the study, the mean DFS and OS were 40 ± 20 months and 46 ± 17 months, respectively.

Among the two Ki-67 groups, there were statistically significant variations in the mean DFS, with the mean DFS being significantly greater in patients with low index than those with high index (64.53 ± 2.48 months [95% CI: 59.67-69.39] and 48.41 ± 4.19 months [95% CI: 40.20-56.63], respectively; $p$=0.001). Additionally, as shown in Figures 2 and 3, when we compared the mean OS in the two groups, we discovered that the OS in the low Ki-67 index group was considerably higher than the OS in the high Ki-67 index group (66.54 ± 1.99 months [95% CI: 62.65-70.43] *vs.* 54.74 ± 3.59 months [95% CI: 47.71-61.77]; $p$=0.002).

In comparison to the patients in the low index group, the incidence of LR and distant metastasis was statistically significantly higher in the high index group [19 (41.3%) *vs.* 8 (12.9%), with a *p*-value of 0.001]. The incidence of bone and lung metastasis was statistically significantly higher in the high index group than in the low index group ($p$=0.001 and 0.038, respectively) while there were no statistically signifi-

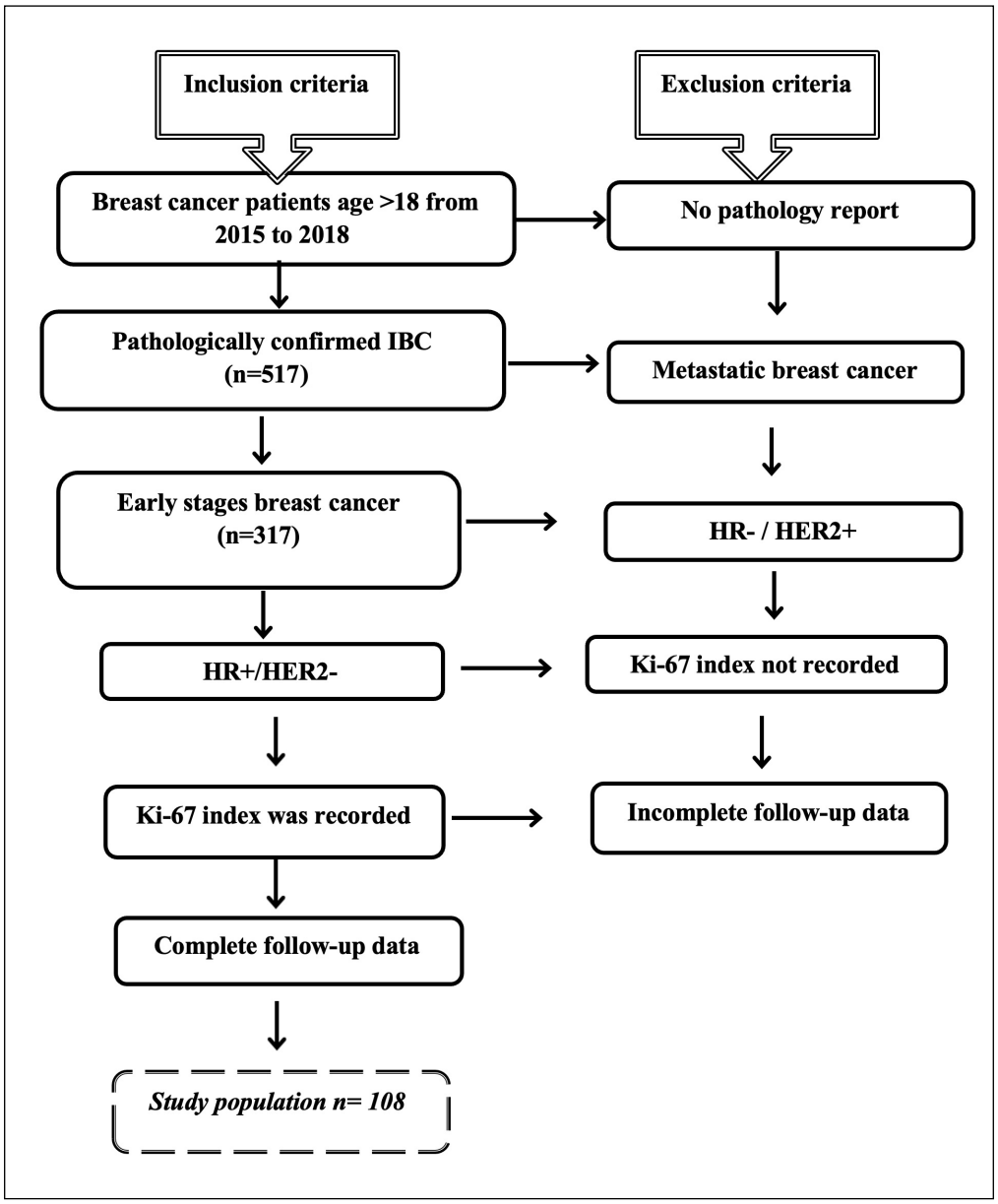


**Figure 1.** Flowchart of the inclusion and exclusion criteria for the study.

cant differences in the incidence of LR, brain, and liver metastasis in either group, as shown in Table 2 and Figure 4. All patients received adjuvant chemotherapy, either in the form of FAC 86 (79.6%), CMF 5, or TAC 17 (15.7%), with no significant difference between the two groups (*p=0.475)*. Additionally, 12 (11.1%) received both letrozole and tamoxifen, with no significant difference (*p=0.726)* between the low and high groups, whereas 47 (43.5%) received tamoxifen and 49 (45.3%) letrozole.

## DISCUSSION

High levels of proliferation in BC are likely to have an aggressive clinical course. Multiple methodological approaches have been used to quantify the evaluation of cellular proliferation in BC cells. The world-wide guideline recommendations for the use of Ki-67 in the prognostic and predictive evaluation of BC remain varied, despite the fact that they all acknowledge that Ki-67 is a prognostic biomarker in BC[37]. Ki-67 is either recommended or taken into consideration for use in the prognostic evaluation of BC patients by the Italian Association of Medical Oncology, the European Group on Tumor Markers, the European Society for Medical Oncology (ESMO) and the National Institute for Health and Care Excellence [38-41].

**Table 1.** Patients and tumor characteristic according to ki-67 index.

| Variables | No. (%) | Ki-67 index Low (n= 62) | Ki-67 index High (n= 46) | *p*-value |
|---|---|---|---|---|
| **Age: (Years)** | | | | |
| **Mean ± SD** | 50.11 ± 10.79 | 50.23 ± 10.20 | 49.96 ± 11.66 | 0.899 |
| **Range** | | 70.0 – 27.0 | 70.0 – 24.0 | |
| **Menopausal status:** | | | | |
| **Pre-menopause** | 44 (40.7%) | 25 (40.3%) | 19 (41.3%) | 0.918 |
| **Post-menopause** | 64 (59.3) | 37 (59.7%) | 27 (58.7%) | |
| **PS:** | | | | |
| **0** | 49 (45.4%) | 28 (45.2%) | 21 (45.7%) | |
| **1** | 41 (38.0%) | 27 (43.5%) | 14 (30.4%) | 0.156 |
| **2** | 18 (16.6%) | 7 (11.3%) | 11 (23.9%) | |
| **Pathology:** | | | | |
| **IDC** | 92 (85.2%) | 49 (79.0%) | 43 (93.5%) | |
| **ILC** | 11 (10.2%) | 9 (14.5%) | 2 (4.3%) | 0.112 |
| **Others** | 5 (4.6%) | 4 (6.5%) | 1 (2.2%) | |
| **T stage** | | | | |
| **T1** | 28 (25.9%) | 22 (35.5%) | 6 (13.0%) | |
| **T2** | 55 (50.9%) | 29 (46.8%) | 26 (56.5%) | 0.043* |
| **T3** | 21 (19.4%) | 10 (16.1%) | 11 (23.9%) | |
| **T4** | 4 (3.7%) | 1 (1.6%) | 3 (6.5%) | |
| **N stage:** | | | | |
| **N0** | 46 (42.6%) | 29 (46.8%) | 17 (37.0%) | |
| **N1** | 31 (28.7%) | 15 (24.2%) | 16 (34.8%) | 0.302 |
| **N2** | 9 (8.3%) | 7 (11.3%) | 2 (4.3%) | |
| **N3** | 22 (20.4%) | 11 (17.7%) | 11 (23.9%) | |
| **Grade:** | | | | |
| **GI** | 15 (13.9%) | 11 (17.7%) | 4 (8.7%) | |
| **GII** | 82 (75.9%) | 47 (75.8%) | 35 (76.1%) | 0.170 |
| **GIII** | 11 (10.2%) | 4 (6.5%) | 7 (15.2%) | |
| **Stage:** | | | | |
| **I** | 14 (13.0%) | 10 (16.1%) | 4 (8.7%) | |
| **II** | 58 (53.7%) | 33 (53.2%) | 25 (54.3%) | 0.485 |
| **III** | 36 (33.3%) | 19 (30.6%) | 17 (37.0%) | |
| **Chemotherapy:** | | | | |
| **FAC** | 86 (79.6%) | 47 (75.8%) | 39 (84.7%) | |
| **CMF** | 5 (4.6%) | 3 (4.8%) | 2 (4.3%) | 0.475 |
| **TAC** | 17 (15.7%) | 12 (19.3%) | 5 (10.8%) | |
| **Hormonal treatment:** | | | | |
| **TAM** | 47 (43.5%) | 25 (40.3%) | 22 (47.8%) | |
| **Letrozole** | 49 (45.3%) | 30 (48.3%) | 19 (41.3%) | 0.726 |
| **TAM+letrozole** | 12 (11.1%) | 7 (11.2%) | 5 (10.8%) | |

**Abbreviations** - **PS:** performance status, **IDC:** invasive ductal carcinoma, **ILC:** invasive lobular carcinoma, **T:** Tumor, **N:** nodal, **G:** grade, **FAC:** 5- Fluorouracil, Adriamycin, and Cyclophosphamide, **CMF:** Cyclophosphamide Methotrexate Fluorouracil, **TAC:** Taxotere, Adriamycin, and Cyclophosphamide.

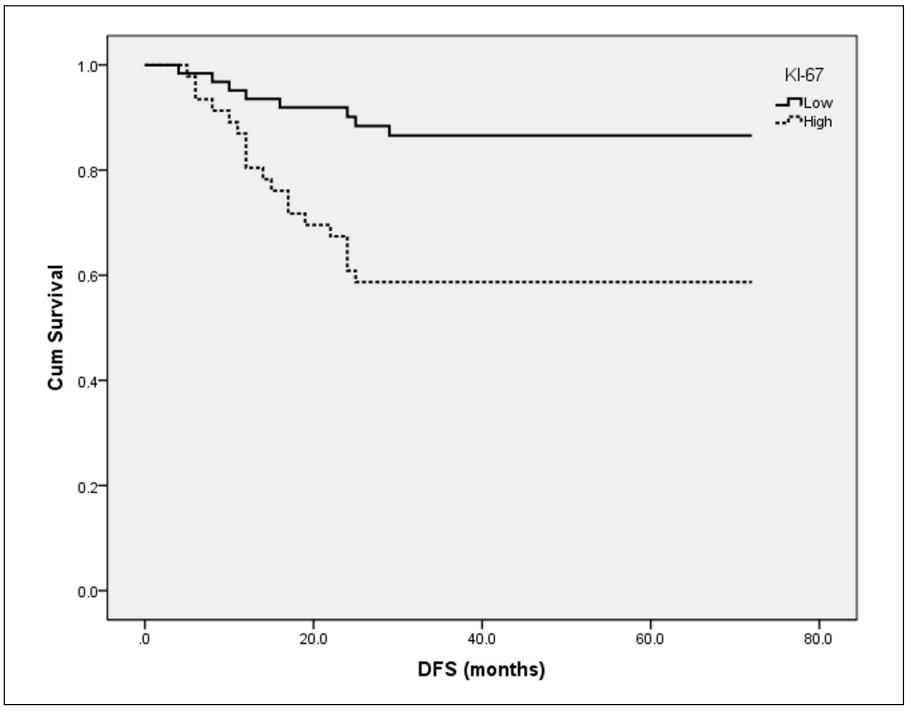


**Figure 2.** Disease-free survival curve according to Ki-67 index.

Neither the American Society of Clinical Oncology (ASCO)[42-45] nor the National Comprehensive Cancer Network (NCCN) supports the use of Ki-67 in the prognostic or predictive evaluation of breast carcinoma. Only in the neoadjuvant situation does the ESMO accept the use of Ki-67 expression as a predictor of response to systemic chemotherapy[46,47]. The American Joint Committee on Cancer (AJCC) advises that "providers and registries should continue to collect and record Ki-67 results" despite the fact that they make no explicit recommendations on the usage of Ki-67 [37,48].

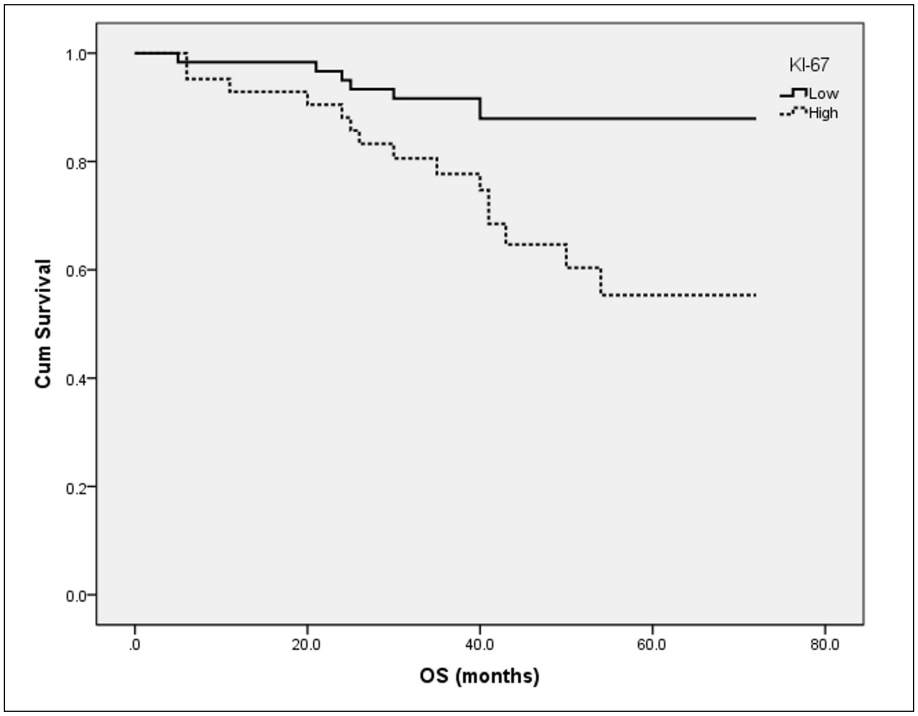


**Figure 3.** Overall survival curve according to Ki-67 index.

**Table 2.** Disease out-come of the patients.

| At the date of cut-off | Total n=108 No (%) | Low Ki-67 index (n=62) | High Ki-67 index (n=46) | *p*-value |
|---|---|---|---|---|
| **Survival** | | | | |
| **Alive** | 80 (74.0%) | 53 (88.5%) | 27 (58.7%) | 0.004* |
| **Missed / died** | 28 (26.0%) | 9 (14.5%) | 19 (41.3%) | |
| **DFS** | | | | |
| **Mean ± SD** | 40 ± 20 | 59.67-69.39 | 40.20-56.63 | 0.001* |
| **95% CI** | | 64.53 ± 2.48 | 48.41 ± 4.19 | |
| **OS** | | | | |
| **Mean ± SD** | 46 ± 17 | 66.54 ± 1.99 | 54.74 ± 3.59 | 0.002* |
| **95% CI** | | 62.65-70.43 | 47.71-61.77 | |
| **LR / Met** | | | | |
| **No** | 81 (75.0%) | 54 (87.1%) | 27 (58.7%) | 0.001* |
| **Yes** | 27 (25.0%) | 8 (12.9%) | 19 (41.3%) | |
| **LR** | 10 (9.3%) | 3 (4.8%) | 7 (15.2%) | 0.094 |
| **Bone met** | 16 (14.8%) | 3 (4.8%) | 13 (28.3%) | 0.001* |
| **Brain met** | 2 (1.9%) | 0 (0.0%) | 2 (4.3%) | 0.179 |
| **Liver met** | 10 (9.3%) | 7 (11.3%) | 3 (6.5%) | 0.512 |
| **Lung met** | 13 (12.0%) | 4 (6.5%) | 9 (19.6%) | 0.038* |

**Abbreviations** - **PS: DFS**: disease-free survival, **OS**: overall survival, **LR**: local recurrence, **Met**: metastasis.

In this work, we investigated its potential as a prognostic and predictive marker. The Ki-67 index and other prognostic markers such as age, menopausal status, PS, pathological type, N stage, tumor G stage, and disease stage did not significantly differ from one another, according to our study. The findings of the N stage were that low N stage was connected with low Ki-67, which was contrary to many earlier researches [49-52].

Regarding tumor G specifically, numerous previous studies discovered a substantial correlation between the high G and the high Ki-67 index [28-30,49-53]. The small number of patients in our study and the brief follow-up time may be to blame for these discrepancies between our results and those of the earlier studies. When comparing patients in the high index group to those in the low index group, we discovered that more patients in the high index group had T stages > T1 ($p$=0.043). These results corroborate those of the other research [49,50,54]. When we looked at the Ki-67 as a predictor, we discovered a significant relationship between the index and the course of the disease, with 88.5% of patients with low index still living at the time the study was stopped, compared to 58.7% of patients with high index ($p$=0.004). These outcomes were consistent with the outcomes of Kanylmaz et al[51]. Additionally, a similar strong link between DFS and OS was discovered, with DFS and OS being significantly lower in patients with high index values than those with low index values ($p$=0.001 and 0.002, respectively). Therefore, our findings support the hypothesis that the Ki-67 biomarker is a reliable indicator of both DFS and OS.

Ki-67 and survival were not correlated in some previous studies, although they were correlated in others [28,49,51,55]. Only the DFS and the Ki-67 index showed a significant association, with OS showing no link, according to Kanylmaz et al [51]. By carrying out a sub-analysis of the recurrences, we discovered that the correlation between the Ki-67 index and the incidence of recurrences was significant only in bone and lung metastasis and non-significant with respect to the other sites of metastasis. We also noticed that the incidence of recurrences was significantly higher in patients with high Ki-67 expression than in patients with low Ki-67 ($p$=0.001).

As we observed, there was no apparent difference between the two groups in terms of the type of the chemotherapy or hormonal therapy which was administered ($p$=0.475 and 0.726, respectively). In contrast to the problematic prognostic indicators of the activity of chemotherapeutic drugs, ER, PR, and HER2 are strong predictors of the hormonal therapy activity [56]. Since most chemotherapy agents require cells to be in the cell cycle, tumors with low proliferated activity; ER, PR positive, HER2 negativity, and low Ki-67 are linked to a lower response to treatment. Based on these results, the question of whether adjuvant chemotherapy should be used remains open [57,58].

Uncertainty persists about the evaluation of Ki-67 despite the published research that examined the predictive function of Ki-67 in BC, in part because the bulk of the investigations was retrospective [59,60]. The routine use of Ki-67 is not recommended because there is no conclusive evidence about the methodology for how to interpret and score Ki-67 levels, or a definition of set Ki-67 cut-off values. A series of suggestions for the global standardization assessment of Ki-67 in BC were published by Dowsett et al [60]. However, the techniques employed to assess the Ki-67 levels varied greatly between laboratories, making it challenging to compare the findings of published investigations [60].

The optimal Ki-67 index cut off value to discriminate between luminal A and B molecular subtypes was found to be 14% by Cheang et al [61]. In the 2011 St. Gallen International Expert Consensus on the Primary Therapy of Early BC, this value was then recommended for clinical usage [48]. The 2013 St. Gallen consensus established a unique cut off value of 20%, in contrast to the recognized 2011 St. Gallen Ki 67 cut off value of 14% to distinguish between the luminal subtypes [48,62]. In order to confirm the 20% Ki-67 cut-off value as the ideal one for stratifying HER-2 enriched and triple negative BC (TNBC) subtypes, randomized prospective studies may be conducted. The prognostic significance of Ki-67 within BC molecular subtypes was assessed in a recent study by Aleskandarany et al [63,64]. The study concluded that the Ki-67 index may differentiate between the luminal subgroups of BC patients and various clinical outcomes. The Ki 67 index, however, showed little effectiveness in stratifying HER-2 enriched and TNBC subtypes because of its higher proliferative activity. Our study has several limitations because few patients were included at the beginning of the trial because Ki-67 was not a routine test [65]. When the test should be performed; if it should come before or after surgery or therapy, its a “cut-off point,” which is still up for debate.

## CONCLUSIONS

The Ki-67 index and T stage are significantly correlated, according to the results of this cohort retrospective single institutional investigation. Additionally, Ki-67 is a potent prognostic and predictive biomarker for DFS and OS in early BC that is ER/PR positive but HER2 negative.

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**Availability of data and materials:**
Data and materials are available on reasonable request contacting the corresponding author.

**Conflict of interest:**
The authors declare no potential conflicts of interest.

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**Ethical Approval:**
This retrospective study was approved by the Ethical Committee of the Faculty of Medicine, Assiut University (IRB local approval number: 17300889).

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