

# Ki-67 Expression as an Indicator of Invasiveness in Patients with Breast Cancer

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## Abstract

**Background:** Tumor markers have a key role in guiding breast cancer management protocols and predicting prognosis. **Objective:** The aim of the study is to explore the role of Ki-67 expression in breast cancer and correlate it to well-known indicators of invasiveness such as age, tumor size, grade, hormonal receptors, and lymph nodes involvement. **Materials and Methods:** This retrospective study was conducted on (214) patients who were newly diagnosed with breast cancer and referred to Azadi Teaching Hospital/Duhok-Iraq, from November 1<sup>st</sup>, 2016, to October 31<sup>st</sup>, 2017. Data regarding patient's demographics, tumor size, histological type and grade, nuclear grade, and lymph node involvement were obtained from medical records. Patients underwent either mastectomy with axillary lymph node dissection or breast conservation surgery. Collected specimens were sent for histopathological examination and immunohistochemistry assessments of estrogen receptor (ER), progesterone receptor (PR), human epidermal growth factor 2 (HER2), and Ki67 expressions. **Results:** Our study showed that histological grade, nuclear grade, mitotic index, and HER2 status were positively correlated to Ki67 index ( $P < 0.05$ ). Furthermore, ER and PR status showed a negative correlation to Ki67 index ( $P < 0.05$ ). Age, tumor size, and the number of lymph nodes involved displayed no significant correlation to Ki67 level ( $P = 0.080, 0.738, \text{ and } 0.888$ ), respectively. **Conclusion:** Results of this study confirmed that Ki67 index are significantly correlated with tumor grade, ER, PR, and HER2 status. However, Ki67 expression association with other clinic-pathological parameters such as age, tumor size, or lymph node involvement is not recognized and requires further studies.

**Keywords:** Biomarkers, breast cancer, immunohistochemistry, Ki-67 antigen

## INTRODUCTION

Breast cancer is the most common cancer in females worldwide. It is the most common cause of cancer and cancer-related morbidity and mortality in Iraqi females according to Iraqi Cancer Registry.<sup>[1,2]</sup> The prognosis is worse in the developing parts of the world and patients with lower educational levels mostly due to late presentation and inadequate follow-up. The important prognostic indicators include age, size of the tumor, axillary lymph nodes involvement, and histological grading of the tumor.<sup>[3]</sup>

Recently, the advancements in biological technology allowed the researchers to have more knowledge of the biological characteristics of the disease and understanding its predictive and prognostic factors to improve the outcome. Various molecular markers have been studied such as estrogen receptors (ERs), progesterone receptors (PRs), human epidermal growth factor 2 (HER2), and Ki-67, to expect the cancer consequences. Expression of these markers affects the

outcome of cancer and clinically has a key role in diagnosis and options of management.

Breast cancer presented clinically in many histopathological types. Invasive breast cancers can be divided into ductal and lobular histologic types. Invasive ductal carcinoma is the most common type of breast cancer, and it forms 50%–70% of all invasive breast cancers. Invasive lobular carcinoma forms around 10% of breast cancers, other types form the remaining percentages.<sup>[4]</sup>

The tumor grade is one of the major prognostic factors for overall survival. Nottingham grading system evaluates the nuclear grade, architectural grade, and mitotic count.<sup>[5]</sup> The

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histopathological grades of breast cancer can be divided into Grade I: Well-differentiated cancer, Grade II: Moderately differentiated cancer, and Grade III: Poorly differentiated cancer. It is generally accepted that higher-grade tumors have increased the risk of lymph-vascular invasion and lower survival rates.<sup>[6]</sup>

Ki67 proliferation protein is a nuclear antigen that was discovered in the early 1980s,<sup>[7]</sup> mainly present in the nucleolar cortex of the nucleolus during interphase of cell division, associated with the periphery of the condensed chromosomes during mitosis.<sup>[8-10]</sup> The half-life of the Ki67 protein range from 1 to 1.5 h.<sup>[11]</sup> Studies have shown that Ki67 is involved in the early steps of polymerase I dependent rRNA formation;<sup>[12]</sup> although Ki67 has a vital role in cell division, its exact function is still not well known. Ki67 expression has different levels throughout the various cell-cycle phases. Cells express the antigen during G1, S, G2, and M phases except the G0 (resting phase).<sup>[13]</sup>

Ki67 expression is low in the G1 and S phases and during mitosis elevated to their maximum levels. Later in the mitotic phase; anaphase and telophase, a rapid reduction in Ki67 levels happen.<sup>[14]</sup> There are 16 rare epithelial subtypes of breast cancer based on the WHO classification.<sup>[15]</sup> Ki67 expression is one of the indicators utilized to characterize the immune profile of these various subgroups. For instance, the lipid-rich breast cancers express high Ki67 levels.<sup>[16,17]</sup> Many studies correlate the clinical value of Ki-67 in breast cancer and prove that it had some prognostic or predictive role in clinical practice.<sup>[18,19]</sup>

The aim of the study is to investigate the role of proliferation antigen Ki-67 expression in breast cancer and correlate it with the indicators of invasiveness of the tumor such as age, tumor size, grade, hormonal receptors, and lymph nodes involvement.

## MATERIALS AND METHODS

This retrospective study was conducted on patients who newly diagnosed with breast cancer referred to breast clinic and Department of Surgery of Azadi Teaching Hospital/Duhok-Iraq, from November 1<sup>st</sup>, 2016, to October 31<sup>st</sup>, 2017. All patients subjected to triple assessments (clinical examination, breast ultrasonography and/or mammography, and histopathological study) confirming the diagnosis and proceed with surgical treatment. Data regarding patient's demographics, tumor size, histological type and grade, nuclear grade, and lymph node involvement were obtained from medical records. Patients underwent either mastectomy with axillary lymph node dissection or breast conservation surgery and sentinel lymph node biopsy. Collected tumor specimens were sent to Duhok Central Teaching Laboratory for histopathological and immunohistochemistry assessments. All tumor specimens were fixed in 10% buffered formalin embedded in paraffin. Histopathological diagnosis and immunohistochemistry interpretation are done by at least two academic pathologists.

This study was reviewed and approved by the Ethical Committee of Kurdistan Board of Medical Specialties/Erbil-Iraq, and written consents were obtained from all the participants. Inclusion criteria included all eligible patients newly diagnosed with breast cancer admitted to Azadi Teaching Hospital for breast surgery. All patients were older than 18 years, and both sexes were included. Exclusion criteria were patients' tumor does not express Ki-67, all breast cancer types other than invasive ductal carcinoma; patients received neoadjuvant chemotherapy, radiotherapy, or any other modality of anticancer therapy before surgery, patients with additional tumors and patient's refusal to be included in the study.

**Histopathological and immunohistochemistry assessment:** For each specimen, five samples of (4 µm) thickness were formed, one of them stained with hematoxylin and eosin stain for histopathological examination, while other samples stained for immunohistochemistry assessment of ER, PR, HER2, and Ki67, which done using standard streptavidin-biotin complex method on automated immunohistochemistry stainer (Dako Autostainer) using reagents and buffers according to manufacturer's guidelines (Dako, Denmark). Negative control samples obtained through replacing the primary antibody with Tris buffer for immune-markers. Positive control samples were obtained using breast cancer sections known to be immune-reactive to ER, PR, and HER2, while human tonsillar sections used as positive control for stained Ki67. Then, slides were examined by ×10 magnification to identified five cellular hot spots which then examined by ×40 magnification. Positive immunoreaction criteria were any brown nuclear stain for ER and PR, dark brown precipitates at cellular membrane for HER2 protein and any immune reactivity for Ki67 considered positive. ER and PR scoring is done based on Allred Score System.<sup>[20]</sup> HER2 scoring was performed according to Dako Scoring System.<sup>[21,22]</sup> Score range from (0) to (+3), both scores (0) and (+1) were considered negative, score (+2) was weakly positive, and score (+3) was strongly positive. Ki67 proliferative index was considered negative when Ki67 tumor cells expression (<14%) and positive if Ki67 tumor expression (≥14%).<sup>[23]</sup>

Tumor staging was based on the 8<sup>th</sup> American Joint Committee on Cancer criteria.<sup>[24]</sup> Histological grade was evaluated by modified Scarff-Bloom-Richardson Scoring System.

## Statistical analysis

Data were analyzed using SPSS Statistics® version 25; IBM® Corporation; USA. Due to the normal distribution of variables, simple regression model and Pearson correlation coefficient test were used to evaluate the association between patient's age, tumor size, histological grade, nuclear grade, ER, PR, HER2, and number of lymph node involved with Ki67 level.  $P < 0.05$  considered statistically significant.

## RESULTS

A total number of (214) patients were included in the study; all patients were females. Mean age of patients was

48.46 ± 11.988 years with a range between 26 and 83 years old. Mean tumor size was 36.10 ± 18.196 mm, minimum and maximum tumor size were 10 mm and 110 mm, respectively. Tumor size divided into groups; T1 (tumor ≤20 mm in its greatest dimension) was found in 45 patients (21%), T2 (tumor >20 mm but ≤50 mm in its greatest dimension) in 140 patients (65.4%), and T3 (tumor >50 mm in its greatest dimension) in 29 patients (13.6%). Lymph node involvement divided into; N0 (no regional lymph node metastases identified) was found in 67 patients (31.3%), N1 (1–3 axillary lymph nodes metastases) found in 61 patients (28.5%), N2 (4–9 lymph nodes metastases) found in 48 patients (22.4%), and N3 (10 or more axillary lymph nodes metastases) was found in 38 patients (17.8%) [Table 1].

ER receptor positive status found in 152 patients (71%) and negative status found in 62 patients (29%). PR receptor positive status was found in 142 patients (66.4%), and negative status was found in 72 patients (33.6%). HER2 status score 0 was found in 67 patients (31.3%), score + 1 was found in 38 patients (17.8%), score + 2 was found in 52 patients (24.3%), and score + 3 was found in 57 patients (26.6%). The histological grade categorized into three groups based on modified Scarff-Bloom-Richardson grading system; Grade 1 which was seen in 17 patients (7.9%), Grade 2 which was seen in 96 patients (44.9%), and Grade 3 which was seen in 101 patients (47.2%). Positive Ki67 index (≥14) was found in 138 patients (64.5%) and negative index (<14) was found in 76 patients (35.5%) [Table 1].

After correlating these variables with the level of Ki67 using simple linear regression model and Pearson correlation coefficient, it was significant in correlation to the ER receptor status ( $P = 0.001$ ), PR receptor status ( $P = 0.001$ ), HER2 receptor status ( $P = 0.021$ ), histological grade ( $P = 0.001$ ), the nuclear grade ( $P = 0.011$ ), and the mitotic index ( $P = 0.009$ ). The correlation was not significant regarding the age of the patient ( $P = 0.080$ ), tumor size ( $P = 0.738$ ), and lymph node involvement ( $P = 0.888$ ) as shown in Table 2.

## DISCUSSION

There are many types of breast cancer with wide range of clinical presentations and prognosis, as the invasive ductal carcinoma is the most common type worldwide, we only included this type in our study, because it will be easier to compare the variable factors for the same histological type of tumor than different histological types, which may negatively affect the results. The age range of our patients was between 26 and 83 years old with a mean age of 48.46 ± 11.988 years old which is approximate to a study done in Iraq and Iran which the mean age was 50.30 ± 9 and 52.8 ± 11.71 years, respectively.<sup>[23,25]</sup>

In most cases of this study, the tumor size was in the T2 group (tumor >20 mm but ≤50 mm); 140 patients (65.4%), comparing to T1 group (tumor ≤20 mm); 45 patients (21%), and T3 group (tumor >50 mm); and 29 patients (13.9%). Which

**Table 1: Distributions of patients related to the clinicopathological parameters (n=214)**

| Main category           | Subcategories | n (%)      |
|-------------------------|---------------|------------|
| Age                     | <35           | 26 (12.1)  |
|                         | 35-50         | 112 (52.3) |
|                         | >50           | 76 (35.5)  |
| Tumor size              | T1            | 45 (21.0)  |
|                         | T2            | 140 (65.4) |
|                         | T3            | 29 (13.6)  |
| Lymph node involvement  | N0            | 67 (31.3)  |
|                         | N1            | 61 (28.5)  |
|                         | N2            | 48 (22.4)  |
|                         | N3            | 38 (17.8)  |
| ER                      | Negative      | 62 (29.0)  |
|                         | Positive      | 152 (71.0) |
| PR                      | Negative      | 72 (33.6)  |
|                         | Positive      | 142 (66.4) |
| HER2 status             | Score 0       | 67 (31.3)  |
|                         | +1            | 38 (17.8)  |
|                         | +2            | 52 (24.3)  |
|                         | +3            | 57 (26.6)  |
| Histopathological grade | 1             | 17 (7.9)   |
|                         | 2             | 96 (44.9)  |
|                         | 3             | 101 (47.2) |
| Ki67 score              | ≥14           | 138 (64.5) |
|                         | <14           | 76 (35.5)  |

ER: Estrogen receptor, PR: Progesterone receptor, HER2: Human epidermal growth factor 2

**Table 2: Correlation between selected variables and Ki67 using simple linear regression test**

| Variable                | B      | 95% CI |        | SE    | P     |
|-------------------------|--------|--------|--------|-------|-------|
|                         |        | Lower  | Upper  |       |       |
| Age                     | 0.062  | -0.008 | 0.131  | 0.035 | 0.080 |
| Tumor size              | 0.018  | -0.088 | 0.124  | 0.045 | 0.738 |
| Lymph nodes involvement | -0.002 | -0.035 | 0.031  | 0.017 | 0.888 |
| ER                      | -0.005 | -0.007 | -0.002 | 0.001 | 0.001 |
| PR                      | -0.005 | -0.008 | -0.003 | 0.001 | 0.001 |
| HER2                    | 0.008  | 0.001  | 0.015  | 0.003 | 0.021 |
| Histopathological grade | 0.010  | 0.007  | 0.014  | 0.002 | 0.001 |
| Mitotic index           | 0.005  | 0.001  | 0.009  | 0.002 | 0.009 |
| Nuclear grade           | 0.004  | 0.001  | 0.007  | 0.002 | 0.011 |

B: Regression coefficient for dependent variable, ER: Estrogen receptor, PR: Progesterone receptor, HER2: Human epidermal growth factor 2, CI: Confidence interval, SE: Standard error

is approximate to studies done in Iraq, Iran, and China which most patients in their studies have tumor size at T2.<sup>[23,25,26]</sup>

Node-negative disease found in 67 patients (31.3%) of our study and the remaining group of patients has a positive node

disease. In a study which included 40 patients, she showed a negative node disease in 11 of her patients (27.5%).<sup>[23]</sup>

The specimens of the majority of patients stained positive for ER and PR receptor (ER  $n = 152$ , 71% and PR  $n = 142$ , 66.4%). Approximate to a study showed 390 of its patients (76%) stained positive for ER and 347, 67.4% for PR, respectively.<sup>[25]</sup>

The histological grade of this study distributed between Grade 2 which found in 96 patients (44.9%) and Grade 3 which found in 101 patients (47.2%). On contrary to studies done on Iraqi and Iranian women which Grade 2 found in 30 patients (75%) and 280 patients (54.58%), respectively.<sup>[23,25]</sup>

Normal breast tissue can express Ki67 which is normally <3%, and higher levels have been detected with increased breast tissue density and precancerous breast lesions.<sup>[27,28]</sup> The tumor was regarded as positive for Ki67 when it is in 14% of the tumor cells or more and considered negative if is detected in <14% of the tumor cells.<sup>[23,29]</sup> In this study, the tumors were positive for Ki67 marker in 138 patients (64.5%) and were negative in the remaining patients. Another study done included 134 patients, they showed that up to 85% of their sample expressed Ki67 markers.<sup>[26]</sup>

Many variables were correlated to the level of Ki67 to see if there is any considerable difference between the positive and the negative groups. There was no significant relation between the level of Ki67 with the age of the patients in our study ( $P = 0.080$ ) which is comparable to another study ( $P = 0.554$ ).<sup>[26]</sup> On the contrary to the result of a study which showed a direct relation with young age groups 30–40 years with a mean Ki67 level of 34.17% and the level was lower in the older age groups 60–70 years.<sup>[25]</sup> A study showed a negative correlation between the tumor size and the Ki67 level ( $n = 123$ ,  $P = 0.063$ ),<sup>[26]</sup> this value is going alongside with our result in regard to the tumor size ( $P = 0.738$ ). The lymph node positive group showed no significant correlation in our study ( $P = 0.888$ ). On the contrary, there was a positive correlation in another study ( $P = 0.025$ ).<sup>[26]</sup>

In our study, the level of Ki67 showed a positive correlation with the levels of the ER and PR receptors ( $P = 0.001$  and  $0.001$ ), respectively, Mirmalek *et al.* showed a positive relation only with ER receptor ( $P = 0.001$ ) and negatively related to PR receptor levels ( $P = 0.058$ ).<sup>[25]</sup> Al-Sarraf and Hussien showed no significant correlation with both ER ( $P = 0.27$ ) and PR ( $P = 0.29$ ).<sup>[23]</sup> HER 2 receptors levels in our patient showed a  $P = 0.021$  which is considered significant, the study of Yang *et al.* showed the same result, while Al-Sarraf and Hussien showed a more considerable correlation ( $P = 0.006$ ), Mirmalek *et al.* showed the same result of Al-Sarraf and Hussien.

Regarding the patients in this study, the nuclear grade of the tumor showed a significant correlation to the Ki67 levels ( $P = 0.011$ ), similar to a study done in Iran ( $P = 0.006$ ).<sup>[25]</sup> Many authors found a direct relationship between the grade of the tumor and the level of the proliferative markers including

Ki67.<sup>[30,31]</sup> The same relation was seen in this study which showed this direct correlation ( $P = 0.001$ ). On the contrary to Finnish study which involved 265 patients showed a negative correlation.<sup>[30]</sup>

### Limitations of this study

More patients from different centers are needed to be included in the study. Besides the absence of few histological parameters indicating distal metastasis.

### CONCLUSION

Ki67 expression index showed a correlation with some of the indicators of invasiveness such as the grade and hormonal receptors, but it showed no correlation with the size, age, and LN involvement. This can be understandable because of other factors which probably interact with the tumor behavior and invasiveness, and the Ki67 is not the sole indicator of invasiveness according to our results.

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### Conflicts of interest

There are no conflicts of interest.

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