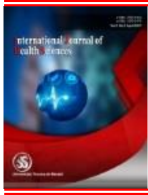

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Ki-67 Expression and its Correlation with Clinicopathological Parameters in Iraqi Breast Cancer Patients

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Abstract

Breast cancer is the most common cancer worldwide and the major cause of cancer-related death among women in both developed and developing countries. In Iraq, breast cancer accounted for 37.9% of all malignant cases in 2020 and 15.3% of cancer-related fatalities. Relevant biomarkers play an important role in predicting prognosis and deciding the most effective therapy for each patient to delay metastases and reduce mortality. Objective: This study aimed to assess the significance of Ki-67 expression as a prognostic biomarker in breast cancer patients as well as investigate the correlations between Ki-67 and their clinicopathological features. Methods: The case-control study comprised sixty newly diagnosed breast cancer patients and ten women with benign breast tumors who served as controls. We assessed the tissue expression level of Ki-67 protein using the immunohistochemistry technique. Results: Our results showed that the median immunohistochemical expression scores of Ki-67 in the newly diagnosed breast cancer group were higher than those of the control group; the difference was significant ($p < 0.001$). The Ki-67 expression score in breast cancer cells increases with tumor size and grade. Ki-67 expression showed a substantial negative correlation with estrogen receptor expression and a significant positive correlation with HER2 expression. Conclusion: Our study confirms that the expression of Ki-67 in breast cancer tissue may be considered an acceptable biomarker and provides additional prognostic information to that gained from traditional prognostic parameters, including pathological grading and tumor size.

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Contents

1 Introduction

Breast cancer (BC) is the most common malignant tumor identified in women. In 2020, it surpassed lung carcinoma as the most commonly diagnosed malignancy, with an anticipated 2.3 million new cases, constituting around 11.7% of all malignancies in the world (Sung et al., 2021). In Iraq, breast cancer is the most frequent malignant tumor in women, accounting for 37.9% of all malignant cases in 2020, 15.3% of deaths, and one-third of recorded malignancies in females of all ages (Globocan, 2020). This malignancy has a significant metastatic potential, which leads to a high fatality rate. Early detection improves outcomes and increases survival rates (Hussein et al., 2022; Ortiz-Abellán et al., 2024). The prognosis and treatment options for the disease are currently customized based on clinical and pathological characteristics, particularly the identification of subtypes using immunohistochemistry (IHC) evaluation of estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor-2 (HER2) (Noske et al., 2020; Tarighati et al., 2023). Despite ongoing efforts, diagnoses and therapies remain incomplete as many patients succumb to recurrent disease. Therefore, it is imperative to improve breast cancer detection and treatment by utilizing novel biomarkers (Ragab et al., 2018). Treatment decisions are a critical step for patients with breast cancer, and one of the most debated metrics is the proliferation marker Ki-67 (Abele et al., 2023; Torlakovic et al., 2024).

Ki-67 is a nuclear protein that binds to DNA (Sobecki et al., 2016), and is found in vertebrates (Booth & Earnshaw, 2017). It is expressed in the cell nucleus throughout all stages of the cell cycle (G1, S, G2, and mitosis), except during the resting phase (G0), where its expression is downregulated to the point of absence. This makes Ki-67 a highly useful and essential biomarker for assessing tumor proliferation and pathology (Zhu et al., 2020; Wu et al., 2019). The Ki-67 antigen was initially discovered in 1983 by Gerdes and colleagues through the use of a mouse monoclonal antibody. The name of the clone was taken from the German city of Kiel and the number assigned to it on a 96-well plate (Zeng et al., 2021; Iqbal et al., 2020). The protein is isoformed at 320 and 359 kDa, resulting from spliced mRNA variations. It is encoded by the human MKI67 gene (Martino et al., 2024;Uxa et al., 2021). Both Ki-67 variants function as surfactants, keeping mitotic chromosomes away once the nuclear membrane breaks down. By interacting with protein phosphatase-1, Ki-67 leads to the development of a perichromosomal protein compartment (Sun & Kaufman, 2018; Booth & Earnshaw, 2017). The amount of Ki-67 present at any given time during the cell cycle is determined by the precise equilibrium between synthesis and degradation. This is evidenced by its very short half-life of 60-90 minutes (Davey et al., 2021). Immunohistochemical evaluation of Ki-67 has become a routine procedure for several forms of cancer due to its strong association with the proliferation of cancer cells (Janssen et al., 2023; Miller et al., 2018). Numerous studies have demonstrated that the expression of Ki-67 is a valuable prognostic indicator in cases of breast cancer (Afkari et al., 2021; Maranta et al., 2020).

Recently, researchers have carried out and assessed multigene platforms to enhance their ability to predict both patient clinical outcomes and response to currently available treatments; however, not all of these platforms have undergone prospective validation, and, more importantly, they are not suitable for all patient subgroups. Moreover, the cost of these platforms is high, and not all countries offer them or local health systems cover them (Barboro et al., 2022). As a result of the lack of prognostic markers, the decision to administer is based on empirical considerations. Furthermore, diagnoses and therapies are imperfect, and many patients die from recurrence (Hussein et al., 2022). This justifies the ongoing pursuit of identifying specific genes or protein candidates that are readily available at a low cost. The objective is to identify novel prognostic markers and enhance the information obtained from clinical and pathological characteristics.

This study aimed to assess the prognostic value of Ki-67 tissue expression in breast cancer patients using immunohistochemistry techniques, to examine the correlations between Ki-67 and various other biomarkers in patients with breast cancer, and to evaluate the relationship between Ki-67 and histological grading.

2 Materials and Methods

A case-control study was conducted from October 2019 to October 2021, involving 70 female patients with breast tumor. Among them, 60 had breast carcinoma and 10 had benign tumor (fibroadenoma), serving as the positive control group. Additionally, 10 healthy women were included as the negative control group. Patients were recruited at the Oncology Teaching Hospital in Baghdad Medical City.

The practical part was conducted in the hospital laboratories, and the other part was conducted in the research laboratories of the Department of Pathology at the College of Medicine/Al-Nahrain University.

The criteria for selection were as follows: (a) A consultant physician evaluated all patients and determined that they had non-metastatic breast cancer, as confirmed by a pathologist. (b) The patient does not have metastatic or recurring breast cancer, and no neoadjuvant therapy has been administered. (c) The patient does not have any systemic illnesses, including hypothalamic or pituitary gland disorders, diabetes, or hypertension. Chronic inflammatory diseases, as well as hepatic, renal, or cardiac failure.

Tissues collection

The number of complete cores taken by the pathologist from each patient was immediately fixed in a 10% formalin solution. Then the specimen was sent to the Pathology Department for histopathological examination and diagnosis. Malignant tissues as well as benign breast tissues were preserved in formalin and embedded in paraffin wax. Sections were stained with routine hematoxylin-eosin staining, diagnosed by pathologists according to the criteria of the World Health Organization, and graded according to the modified Scarff-Bloom and Richardson method.

The selected patients were subjected to breast surgery, which was either lumpectomy or mastectomy. All patients had undergone clinical examinations and routine laboratory examinations: a complete blood count, liver and renal function tests, chest X-rays and mammograms, breast and abdominal ultrasonography, and a bone scan. The histopathological reports revealed the patient's age, tumor type and size, grade, nodal status, estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor-2 (HER2) status.

Expression of Ki-67 in tissue

Ki-67 analysis was performed by immunohistochemical staining, and assessing the proportion of malignant cells showing positive staining for the nuclear antigen Ki-67 was conducted quantitatively and visually under light microscopes. The Ki-67 score is presented as the percentage of immunostained nuclei relative to the total number of nuclei in tumor cells, regardless of the intensity of immunostaining.

The approximate proportion of stained cells in each tissue was determined by categorizing the proportion score as one of the following: 0: <1%, 1: 1–15%, 2: 16–29%, 3: >30%. These scores corresponded to the level of positivity: 1: low positivity, 2: moderate positivity, and 3: high positivity, following the guidelines of the St. Gallen International Consensus of Experts (Aman et al., 2019; Goldhirsch et al., 2011). The positive cut-off level for Ki-67 was ≥ 2.

Statistical analysis

Data were collected, summarized, analyzed, and presented using the Statistical Package for Social Sciences (SPSS) version 23 and Microsoft Office Excel 2010. Due to the skewed distribution of the data as indicated by statistical analysis, non-parametric tests were chosen. Categorical and continuous variables were examined using chi-square, Mann-Whitney U tests, and Spearman correlation tests.

Qualitative (categorical) variables were expressed as numbers and percentages, whereas quantitative (numeric) variables were first evaluated for normality distribution using the Kolmogorov-Smirnov test, and then accordingly, normally distributed numeric variables were expressed as mean (an index of central tendency) and standard deviation (an index of dispersion), while those numeric variables that are not normally distributed were expressed as median (an index of central tendency) and interquartile range (an index of dispersion). The level of significance was considered at a P-value of equal to or less than 0.05. The level of high significance was considered at a P-value of equal or less than 0.01.

3 Results and Discussions

3.1 Results

This study included data from sixty patients who met the inclusion criteria. The clinicopathological characteristics of women with breast carcinoma are demonstrated in Table 1. The mean age of all patients was 50.02 ± 10.68 years (extremes: 32–75 years). Among the 60 patients, 53.3% were pre-menopausal compared to 46.7% post-menopausal. According to breast cancer type, 88.3% were invasive ductal carcinoma, 10.0% were invasive lobular carcinoma, and a single case (1.7%) was mixed invasive ducal and lobular carcinoma. Grade II tumors were 61.6%, followed by grade III (21.7%) and grade I (16.7%). The positivity rates for ER, PR, and HER2 were 68.3%, 55%, and 31.7%, respectively.

Table 1
Comparison of mean age and frequency distribution according to age among study groups

Characteristic	Breast Carcinoma; *n* = 60
Menopause status	
Pre-menopause	32 (53.3 %)
Post-menopause	28 (46.7 %)
Type	
IDC, *n* (%)	53 (88.3 %)
ILC, *n* (%)	6 (10.0 %)
IDC and ILC	1 (1.7 %)
Grade	
I, *n* (%)	10 (16.7 %)
II, *n* (%)	37 (61.7 %)
III, *n* (%)	13 (21.7 %)
Tumor size	
T1, *n* (%)	12 (20.0 %)
T2, *n* (%)	38 (63.3 %)
T3, *n* (%)	10 (16.7 %)
Lymph node status	
Negative, *n* (%)	19 (31.7 %)
N1, *n* (%)	23 (38.3 %)
N2, *n* (%)	14 (23.3 %)
N3, *n* (%)	4 (6.7 %)
Estrogen receptor	
Negative, n (%)	19 (31.7 %)
Positive, n (%)	41 (68.3 %)
Progesterone receptor	
Negative, n (%)	27 (45 %)
Positive, n (%)	33 (55 %)

Characteristic	Breast Carcinoma; *n* = 60
HER2	
Negative, n (%)	41 (68.3 %)
Positive, n (%)	19 (31.7 %)

Immunohistochemical expression of Ki-67

The distribution of the Ki-67 labeling index as measured by the percentage of positively stained cells is shown in Figs. 1–4. Positive immunoperoxidase reaction in the Ki-67 antibody-stained sections was confined to the nuclei of carcinoma cells (Fig. 1-3) and was found to be expressed in 59 out of 60 cancer tissue samples studied, where fifteen (25%) achieved high positivity cells (>30%), 26 (43.3%) showed moderate positivity (16–29%), whereas low positivity cells (1–15%) were detected in 18 cases (30%).

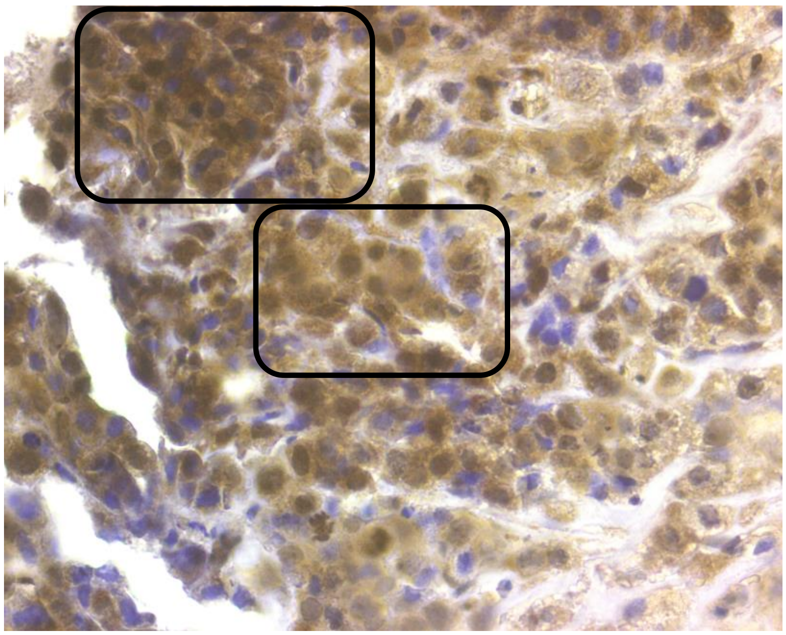

Figure 1. Nuclear staining for Ki-67 with a value >50 (high) is dark-brown in the case of invasive duct carcinoma with grade III (×40)

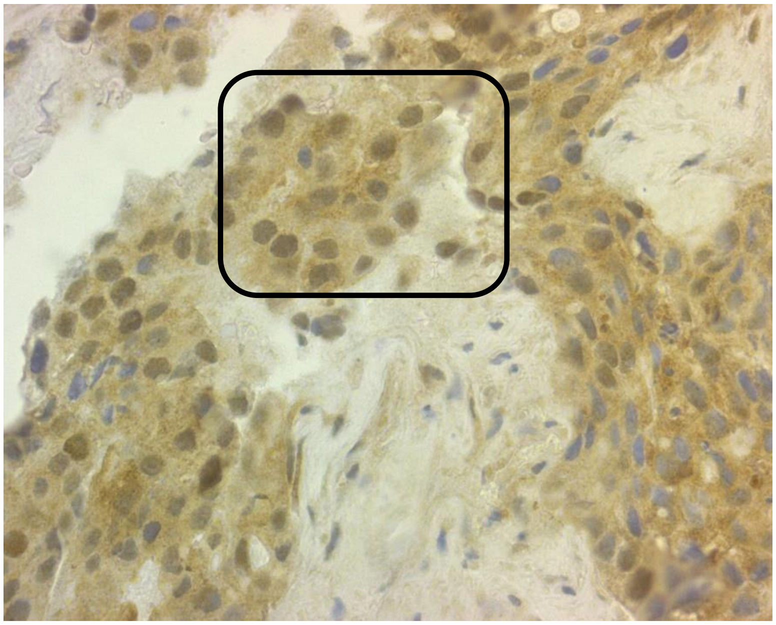

Figure 2. Nuclear staining for Ki-67 with a value >30 (high) was dark brown in a case of invasive duct carcinoma with grade II (×40)

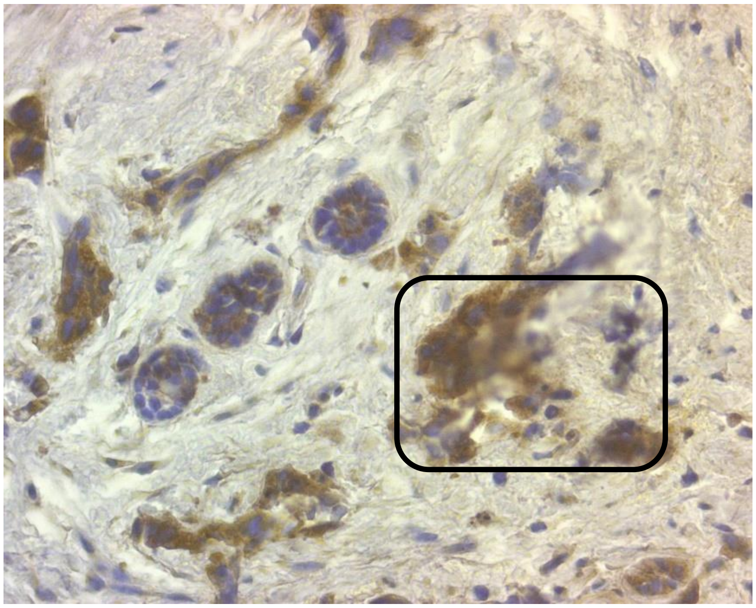

Figure 3. Nuclear staining for Ki-67 with a value < 16 (low) was dark-brown in a case of invasive duct carcinoma with grade I (×40)

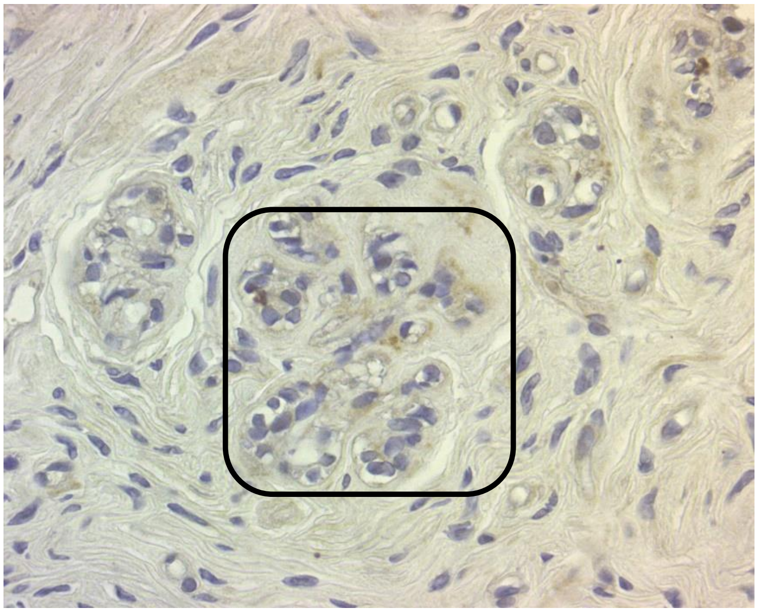

Figure 4. Nuclear staining for Ki-67 with a value of very low was dark-brown (blue nucleus) in a case of benign breast tumor (fibroadenoma) (×40)

IHC-expression scores of Ki-67 in the control (benign) group and the group of women with breast carcinoma are shown in Table 2. The median expression score of the breast cancer group was higher than that of the control group, 2 versus 1, respectively, and the difference was significant (p < 0.001).

Table 2
Immunohistochemical expression scores of Ki-67 in the control and breast carcinoma groups

Marker	Control group	Newly diagnosed BC	*p*
Ki-67			
Median (IQR)	1 (0)	2 (2)	<0.001 M **
Range	1 - 1	1 - 3	

n: denotes the number of cases; **BC**: breast carcinoma; **IQR**: denotes inter-quartile range; **M**: denotes the Mann Whitney U test; **NS**: denotes not significant at $p > 0.05$; **: denotes significant at $p \leq 0.01$.

Correlations of immunohistochemical expression of Ki-67 to clinicopathological characteristics

Correlations of IHC-expression of ki-67 with clinicopathological characteristics of patients with breast carcinoma are shown in Table 3. Regarding the menopause status, tumor type, grade and size of the tumor, and nodal status, ki-67 showed a significant positive correlation to both grade and tumor size. Regarding hormonal status, Ki-67 showed a significant negative correlation to ER and a significant positive correlation to Her2neu.

Table 3
Correlations between IHC-expression of ki-67 and clinicopathological characteristics of breast carcinoma patients

Clinicopathological characteristics	Ki-67	
	r	*p*
menopause status	-0.249	0.304
Type	-0.174	0.184
Grade	0.681	< 0.001 **

Clinicopathological characteristics	Ki-67	
	r	*p*
Tumor size	0.361	0.005 **
Lymph node status	0.144	0.273
ER	-0.254	0.050 *
PR	-0.218	0.095
Her2neu	0.512	< 0.001 **

*: denotes statistically significant at $p \leq 0.05$; **: denotes statistically significant at $p \leq 0.01$

3.2 Discussion

Proliferation is one of the most important aspects of all cancer types, including breast cancer; thus, its study can provide valuable information about the disease status (Ács et al., 2017; Cianfrocca & Goldstein, 2004). Ki-67 is an indicator of cell proliferation that is elevated in a variety of cancers (Wang et al., 2021; Zhou et al., 2024).

The expression of Ki-67 was assessed in the tissues of patients who were recently diagnosed with breast cancer (BC) and benign conditions. The median score of Ki-67 expression in the BC group was found to be greater than that of the control group, as indicated in Table 2. The BC group showed a significant inverse relationship between Ki-67 expression and ER, as well as a remarkably significant positive correlation with HER2, as indicated in Table 3.

The findings of this study were consistent with previous research conducted by Ahmed et al. (2018) and Ragab et al. (2018), which found a significant negative correlation between the Ki-67 index and ER and PR, however the data showed a positive correlation with HER2 status. However, this contradicts the results of a study conducted by Aman et al. (2019), which demonstrated a substantial positive association between the ki-67 index and ER but no significant association with HER2. Furthermore, as shown in Table 3.8, this investigation showed a significant positive association with grade and tumor size, but no significant association with lymph node status. Ragab et al. (2018), found that the immunohistochemical Ki-67 level rises when the tumor size, grade, and lymph node status increase. Ahmed et al. (2018), found a strong correlation between the Ki-67 index and the tumor grade, but no significant correlation with tumor size or nodal status. There is a correlation between Ki-67-labeled tumor growth and tumor aggression, which is determined by cancer grade and stage (Jakobsen & Sørensen, 2013). Mitotic activity is one of the three primary components of the BC grade system, as defined by Elston and Ellis' criteria (1991). It represents the rate of malignant cell growth, along with the Ki-67 proliferative index, which is seen throughout all stages of the cell cycle, without phase G0. A greater association between Ki-67 and high grades indicates a more aggressive cancer with a higher probability of recurrence and metastasis (Aman et al., 2019). Previous studies have demonstrated the predictive value of Ki-67, a key biomarker of cell proliferation, in a variety of cancers originating from organs and tissues, including breast cancer. A high Ki-67 protein expression level was associated with a poor prognosis (Wu et al., 2024; Wu et al., 2019). The evolution of breast cancer generally involves changes in the expression of PR, ER, and HER2 receptor status, characterized by a decrease in ER and PR expression and an increase in HER2 expression (Begum et al., 2012). Following the current results, other studies found that a Ki-67 expression was directly associated with pT, tumor grade, and inversely associated with ER and PR expression (Viale et al., 2019; Al-Nuaimi et al., 2020), and directly proportional to the expression of HER2 (Hashmi et al., 2019). This finding was consistent with the fact that recurring cancers had much worse survival rates, regardless of whether the primary tumors showed high or low Ki-67 levels. Furthermore, a meta-analysis of 12,155 individuals found that Ki-67 positivity indicates a greater probability of recurrence and a worse prognosis in early breast cancer patients (Yerushalmi et al., 2010). In contrast, other studies reported that tumor size and HER2 status do not affect the patients' outcomes, while patients with ER positives show higher expression for Ki-67 (Kamranzadeh et al., 2019).

4 Conclusion

The results of this study confirm that the expression of Ki-67 in tissue may be considered an acceptable biomarker and provide additional prognostic information to that gained from traditional prognostic parameters, including pathological grading, tumor size, and involvement of lymph nodes. We recommend prioritizing the standardization of Ki-67 evaluation to prevent any conflicting outcomes in Ki-67 results.

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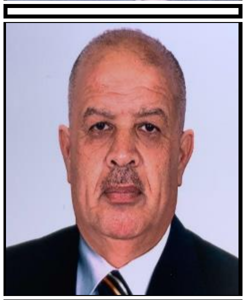

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