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Research Article

Exploring the Relationship Between Ki-67 Immunohistochemical Expression and Key Histopathological Parameters in Breast Cancer

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Abstract

Background: Breast cancer is the most common cancer in women around the world. Ki-67 is a nuclear protein that is linked to cell growth. It has become an important biomarker for predicting and diagnosing breast cancer. The goal of this study was to find out if there was a link between Ki-67 immunohistochemical expression and important clinicopathological factors in breast cancer patients, such as their age, work, histopathological type, tumor grade, and HER-2 status.
Methodology: This study was conducted on a sample of 40 cases of breast cancer diagnosed in women from Najaf Governorate A cross-sectional observational research was performed on breast cancer tissue samples to assess Ki-67 expression using immunohistochemistry labeling. Patient demographic information and tumor attributes were documented. Statistical analysis was conducted to determine significant connections ($P \leq 0.05$).
Result: The study indicated that 85% of breast cancer cases demonstrated positive Ki-67 immunohistochemistry expression, primarily in patients aged 50–59 years and in housewives. Invasive ductal carcinoma was the predominant histologic type linked to Ki-67 positive. Moreover, elevated Ki-67 expression was noted in Grade II and III tumors and was more common in HER-2 negative patients. Statistically significant correlations were seen between Ki-67 expression and all assessed clinicopathological characteristics ($P < 0.05$).
Conclusion: Ki-67 immunohistochemistry expression demonstrates a substantial correlation with essential histological and clinical characteristics, indicating its prospective value as a prognostic marker in breast cancer. Additional research is required to confirm its function and improve personalized therapy approaches.

1. Introduction

According to [1, 2], breast cancer is one of the most prevalent forms of cancer in females, with 2.3 million new cases and 585,000 deaths associated with it each year. A cluster of cancerous cells, also known as a tumor, can invade nearby tissues, cause damage, and potentially spread to other parts of the body. However, abnormal changes in breast cells can also result in non-cancerous conditions such as atypical hyperplasia, cysts, or benign tumors such as intraductal papillomas [3]. Breast cancer begins in the cells of the breast, where it can spread across the body. Sustained proliferative signaling is one of the characteristics that distinguish breast cancer from other types of cancer. This kind of signaling may be seen in living organisms by the detection of proliferation markers such the Ki-67 protein, which is produced in the nucleus throughout the G1, S, and G2 phases of the cell cycle as well as during mitosis [4]. There is a close connection between the nuclear

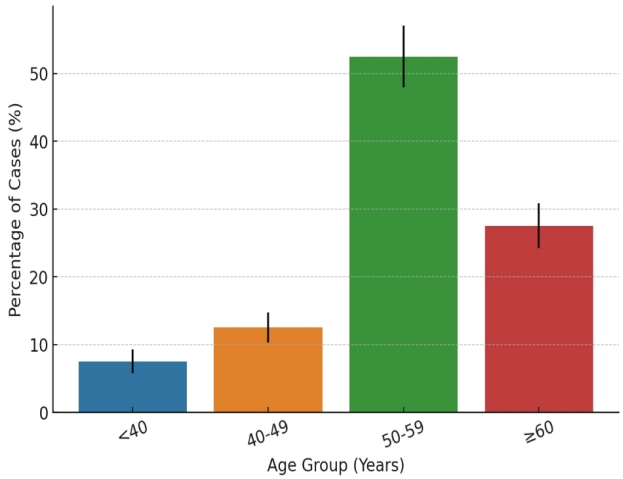

Figure 1: Breast Cancer Cases by Age Group (%)
Source: Output generated by IBM SPSS Statistics, version 26.0, based on study data collected in 2023.

non-histone protein known as Ki-67 and the process of cell proliferation. Ki-67 is expressed throughout the G1, S, G2, and M phases of cell division, but it is not present during the G0 phase [5, 6]. A major biomarker for determining the aggressiveness of tumors and predicting the outcomes of breast cancer, Ki-67 has become an important biomarker due to the critical role it performs. In female breast cancer patients, the purpose of this research was to investigate whether or not there is a link between the expression of Ki-67 immunohistochemistry and factors such as age, occupation, tumor grade, histological type, and HER-2 status.

2. Materials and Methods

This study was conducted on a sample of 40 cases of breast cancer diagnosed in women from Najaf Governorate.research Design: A cross-sectional observational research. Sample Collection: Forty breast cancer tissue samples, fixed in formalin and embedded in paraffin, were procured from Al-Sadr Teaching Hospital and private laboratories in Najaf between July 2024 and May 2025. Eligible participants consisted of females aged 30 to 70 years with histopathologically confirmed breast cancer.

Immunohistochemistry

IHC, or immunohistochemistry, is a basic diagnosis tool that finds certain proteins inside cells by using interactions between antigens and antibodies. Because it is accurate and can tell the difference between protein types, it is used a lot in cancer studies [7]. The process is very careful and includes steps like deparaffinization, rehydration, and antigen retrieval using solutions like citrate buffer (pH 6.0) or Tris-EDTA (pH 9.0). Next, hydrogen peroxide and protein-blocking buffer are used to stop nonspecific binding [8, 9], and [10]. Next, primary antibodies are added. After washing, horseradish peroxidase (HRP)-conjugated secondary antibodies are added. Adding the chromogen 3,3'-diaminobenzidine (DAB) makes the target protein stand out as a dark solid [11, 12]. At the end of the process, hematoxylin counterstaining, dehydration, and mounting the slide for microscope examination are done. IHC is often used to find ER, PR, and HER2 hormonal receptors in breast cancer, as well as CD3+, CD8+, and FoxP3+ markers of immune cell invasion [7, 13]. The Allred scoring method is used to rate ER and PR stains. It takes the proportion score (0–5) and the strength score (0–3) and adds them together to get a score from 0 to 8. Scores from 0 to 8 are categorized as either negative (0–2) or strongly positive (7–8) [14]. We know that HER2 is present if more than 30% of the tumor cells show 3+ membrane stains. The test is not positive if more than 10% of the cells show 2+ stains. A type of cancer that doesn't meet these criteria is known as triple-negative [15]. A Ki-67 monoclonal antibody was used to color slices of tissue. Nuclear staining showed positive expression in more than 20% of tumor cells. Expert doctors looked at the slides at 10x and 40x magnification.

Statistical Analysis

SPSS version 26 was used in order to carry out the data analysis. The evaluation of the connection between Ki-67 expression and clinicopathological variables was conducted by means of the chi-square test, with statistical significance being regarded as a value of P greater than 0.05.

3. Results

Of the 40 cases, 34 (85%) demonstrated positive Ki-67 expression, whereas 6 (15%) were negative. The predominant age group of Ki-67-positive cases were individuals aged 50–59 years (47.5%), followed by those over 60 years (27.5%). The largest group exhibiting positive Ki-67 expression were housewives (47.5%), followed by employees (27.5%). Histopathological Type: Invasive ductal carcinoma (IDC) exhibited the highest Ki-67 positivity at 67.5%. Tumor Grade: Grade II tumors exhibited the greatest Ki-67 positivity at 42.5%, followed by Grade III at 27.5%. HER-2 Status: Ki-67 positivity was more common in HER-2 negative cases (57.5%) than in HER-2 positive cases (27.5%). All differences were statistically significant ($P \leq 0.05$). See more in the table 1 and figures 1-6.

Table 1: Demographic Characteristics of Participants and Ki-67 Expression in Breast Cancer

Variables	**Immunohistochemical biomarkers expression Ki-67No(%)**	
	+ve	-ve
Age (years)		
<40	2(5)C,a	1(2.5)C,b
40-49	2(5) C,b	3(7.5) A,a
50-59	19(47.5) A,a	2(5) B,b
>60	11(27.5) B,a	0(0) D,b
Total	34(85)a	6(15)b
Job		
Self-employment	4(10)C,a	2(5)A,b
Employee	11(27.5)B,a	2(5)A,b
Housewife	19(47.5)A,a	2(5)A,b
Total	34(85)a	6(15)b
Histopathological type		
Ductal Carcinoma In Situ - DCIS	1(2.5)C,a	1(2.5)B,a
Invasive ductal carcinoma (IDC)	27(67.5)A,a	5(12.5)A,b
Invasive lobular carcinoma (ILC)	5(12.5)B,a	0(0)C,b
Medullary Carcinoma	1(2.5)C,a	0(0)C,b
Total	34(85)a	6(15)b
Tumor grade		
I	6(15)C,a	1(2.5)B,b
II	17(42.5)A,a	4(10)A,b
III	11(27.5)B,a	1(2.5)B,b
Total	34(85)a	6(15)b
HER-2		
Positive	11(27.5)B,a	2(5)B,b
Negative	23(57.5)A,a	4(10)A,b
Total	34(85)a	6(15)b

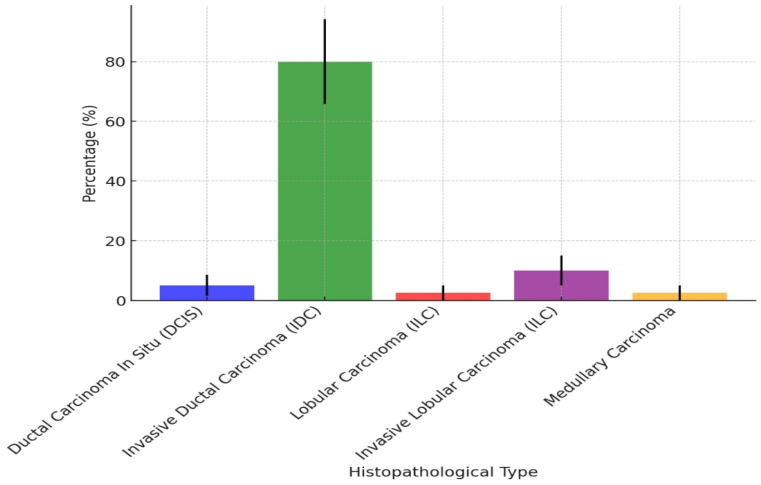

Figure 2: Distribution of Breast Cancer Histopathological Types (%)
Source: Output generated by IBM SPSS Statistics, version 26.0, based on study data collected in 2023.

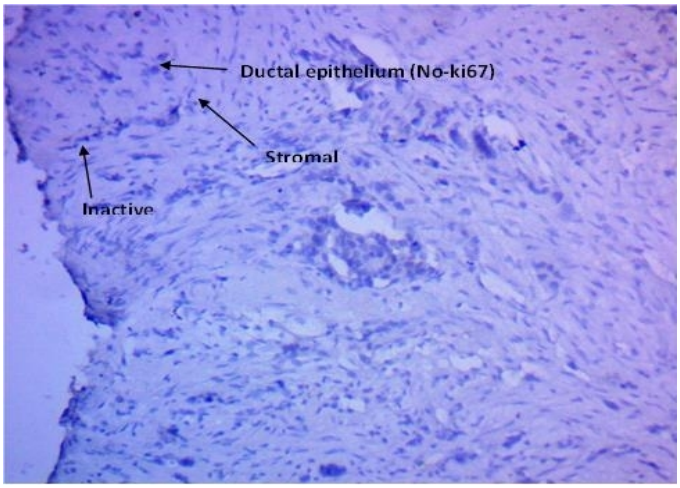

Figure 4: Negative Ki-67 IHC Control in Normal Breast Tissue.

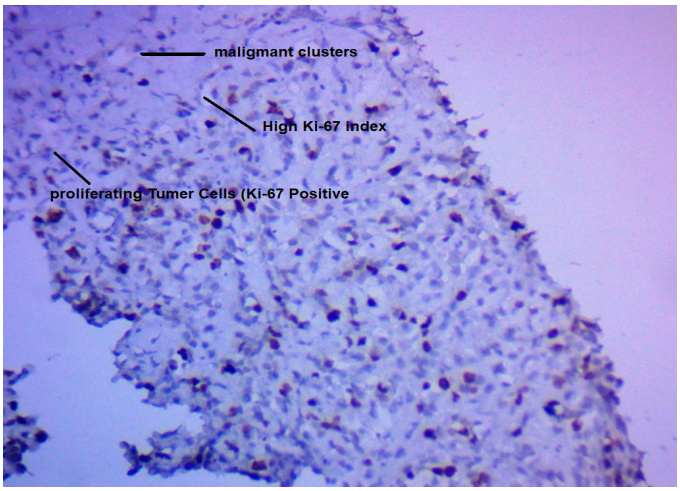

Figure 5: Breast cancer cells that show positive Ki-67 expression (IHC).

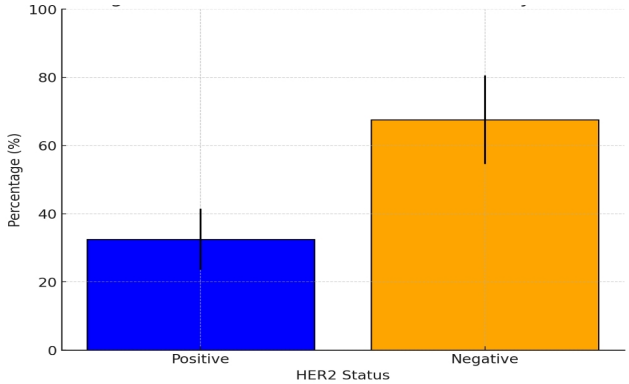

Figure 3: HER2 Status Distribution in Breast Cancer Cases (%)
Source: Output generated by IBM SPSS Statistics, version 26.0, based on study data collected in 2023.

This histological image represents a section of normal breast tissue stained with Immunohistochemistry (IHC) for the Ki-67 protein, serving as a negative control. Ki-67 is a protein found in the nucleus that is only found in cells that are growing (G1, S, G2, M phases) and not in cells that are not growing (G0 phases).

In normal breast tissue, the proliferative activity is low, and thus Ki-67 expression is minimal. Key observations include:

- Ductal Epithelium (No Ki-67): The epithelial lining of ducts shows no brown nuclear staining, indicating the absence of active cell proliferation.

- Inactive Nuclei: The nuclei appear blue, showing no evidence of Ki-67 expression.

- Stromal Tissue: Connective tissue surrounding the ducts is devoid of proliferation, as expected.

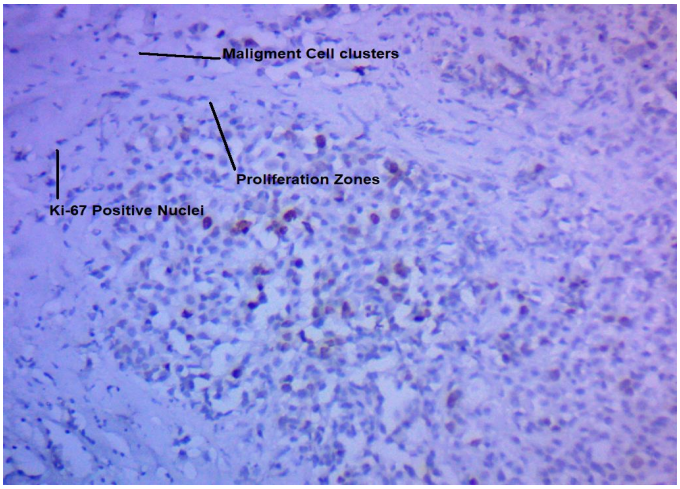

Figure 6: Positive Ki-67 Expression in Breast Carcinoma (IHC) - Case 5.

4. Discussion

This study shows that there is a strong link between Ki-67 expression and several clinical and molecular features of breast cancer. The findings correspond with previous studies demonstrating that elevated Ki-67 expression is associated with higher tumor grade and enhanced proliferative activity [16, 17]. The elevated incidence of Ki-67 positive in IDC cases supports its established aggressive clinical characteristics [9]. Notably, heightened Ki-67 expression was more pronounced in HER-2-negative patients, in contrast to multiple prior studies associating Ki-67 with HER-2 positive [18, 19]. This gap may be due to sample size, population variations, or tumor heterogeneity. The prevalence of Ki-67 positive among middle-aged patients and housewives may indicate biological and social factors affecting tumor biology, necessitating additional research. Ki-67's dynamic function throughout cell cycle phases underscores its value as a proliferation marker and prognostic instrument. Due to its prognostic importance, Ki-67 evaluation should be incorporated into standard pathology assessments to enhance breast cancer classification and inform treatment choices.

The current study revealed that **85% of breast cancer cases exhibited positive Ki-67 immunohistochemical expression**, indicating a high proliferative index among the tumor samples. The expression of Ki-67 varied significantly across clinical and pathological variables such as age, occupation, histopathological type, tumor grade, and HER2 status, demonstrating its relevance as a biological marker for tumor aggressiveness.

Age-related differences showed the highest Ki-67 expression in the 50–59-year age group (47.5%), followed by those older than 60 years (27.5%). This finding is consistent with the work of [20], who reported that Ki-67 expression increases with age due to the accumulation of genetic alterations and the transition toward more aggressive tumor phenotypes in older patients.

In terms of **occupation**, housewives showed the highest Ki-67 expression (47.5%), followed by employed women (27.5%). While occupation is not a widely studied variable in breast cancer immunohistochemistry, this observation may reflect lifestyle or health access disparities and highlights a potential area for further research.

Regarding **histopathological type**, the highest Ki-67 expression was observed in **invasive ductal carcinoma (IDC)** (67.5%), which aligns with [21], who emphasized that IDC is commonly associated with high proliferative activity and poorer prognosis due to elevated Ki-67 levels.

Concerning **tumor grade**, grade II tumors showed the highest Ki-67 expression (42.5%), followed by grade III (27.5%). These findings support the well-established relationship between high Ki-67 index and advanced tumor grades, which has been documented by [22].

In terms of **HER2 status**, tumors with **HER2-negative** expression had a higher proportion of Ki-67 positivity (57.5%) compared to HER2-positive cases (27.5%). This outcome is in agreement with the findings of [23], who noted a complex interaction between HER2 and Ki-67 expression patterns, with some HER2 tumors exhibiting higher proliferation rates independent of HER2 signaling pathways.

5. Conclusion

This study shows that Ki-67 is still a useful measure for detecting breast cancer growth and predicting how the cancer will progress. There was a strong link between the immunohistochemical expression of Ki-67 and a number of important clinicopathological factors. These included age, work, tumor grade, histological type, and HER-2 status. These links show how important Ki-67 is for making decisions about treatment, figuring out risk, and managing each patient individually. Also, the interesting connection between Ki-67 expression and sociodemographic factors like work points to an area that hasn't been looked into much but needs more research. So, it is suggested that more large-scale, joint studies be done to confirm these results and learn more about the molecular processes that cause Ki-67 to have different prognostic values in different types of breast cancer.

Advantages and Disadvantages: Advantages

Application of advanced immunohistochemistry techniques. Association with several clinicopathological factors.

Constraints

The limited sample size constrains the generalizability of the findings. Absence of molecular subtype categorization (e.g., Luminal A, B, Basal-like). Lack of survival or follow-up statistics.

Suggestions

Subsequent investigations ought to involve bigger, multicenter cohorts with molecular subtyping and survival analysis to thoroughly evaluate the prognostic significance of Ki-67 and enhance individualized treatment strategies.

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