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ORIGINAL ARTICLE

Correlation of Ki67 expression with estrogen receptor (ER) and progesterone receptor (PR) status in breast cancer

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ABSTRACT

Aim To determine whether Ki67 can be predicted through its correlation with the estrogen receptor (ER) and progesterone receptor (PR), to identify breast cancer subtypes and evaluate cell proliferation level and prognosis.

Methods Patient clinical data were collected from medical records and pathology laboratory reports at Teaching Hospital, Faculty of Medicine, Universitas Sumatera Utara. Ki67 expression, as a proliferation marker, was measured and detected using immunohistochemistry (IHC). Ki67 data were categorized into two groups: <20% (low proliferation) and >20% (high proliferation). Statistical analysis was conducted by calculating the frequency distribution and percentage of receptor status and using the $\chi 2$ test.

Results The study revealed an inverse relationship between ER/PR status and Ki67 (<0.001). Low Ki67 levels were generally associated with positive ER/PR status, while high Ki67 levels showed increased frequency in ER/PR- negative cases.

Conclusion: Ki67 expression can be determined based on the frequency of ER/PR status, however, these markers have distinct roles in the management of breast cancer patients.

Keywords: biomarkers, immunohistochemistry, Ki67, prognosis, receptors

INTRODUCTION

Breast cancer remains the leading cause of cancer-related morbidity and mortality among women worldwide (1). In 2020 breast cancer was the most common type of cancer with the highest mortality rate for women in Indonesia (1). Factors associated with this cancer include obesity, age at first childbirth, breastfeeding history, and age at menarche (2). Breast cancer growth is caused by the proliferation of cancer cells that is much faster than normal ductal epithelial cells of the breast. This proliferation is autonomous and not controlled by bodily signals (3). Although all breast cancer cells have a high growth rate, the level of proliferation varies between individuals. In cases of low proliferation, cancer growth tends to be slow, whereas high proliferation leads to rapid growth and metastasis to other organs (3).

Breast cancer can be molecularly classified based on immunohistochemical indicators such as estrogen receptor (ER), progesterone receptor (PR), and the cell proliferation index (Ki67) (4). Estrogen and progesterone receptors are capable of determining prognostic factors, although their significance primarily lies in predicting responses to endocrine therapy (5). Several studies have shown an inverse relationship between Ki67 and positive ER/PR status, where an increase in Ki67 corresponds to a decrease in ER/PR levels (5–7).

Ki67 is a nuclear protein that indicates cell proliferation and is often used to assess tumour aggressiveness in breast cancer (8). Ki67 protein expression reflects tumour cell activity and strongly correlates with tumour progression, metastasis, and prognosis (4). In luminal breast cancer subtypes (ER/PR positive), Ki67 helps distinguish between Luminal A (low proliferation, better prognosis) and Luminal B (high proliferation, worse prognosis) subtypes (6,8). This makes it an important biomarker for patient risk stratification and determining appropriate therapy (4). Biomarkers are biological molecules found in blood, other body fluids, or tissues that serve as indicators of normal or abnormal processes, specific conditions, or diseases (9). In breast cancer, biomarkers are crucial for diagnosis, prognosis, and determining proper therapy (10).

Immunohistochemistry (IHC) examination is an important method for diagnosing and accurately determining breast cancer types (6). The IHC helps identify the expression of receptor proteins, ER, and PR, which play roles in planning appropriate therapy (6,11). In general, hospitals in the Medan area only examine ER and PR status in breast cancer evaluations. However, Ki67, which is an important biomarker in assessing cell proliferation, has not yet been assessed.

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This study aimed to determine whether Ki67 can be predicted through its correlation with ER and PR, to identify breast cancer subtypes and evaluate cell proliferation levels and prognosis, and if Ki67 can be predicted solely by assessing ER/PR status, without the need for specific staining.

PATIENTS AND METHODS

Patients and study design

In this retrospective study clinical data of breast cancer patients were collected from medical records and the Pathology Laboratory archives at the Teaching Hospital, Faculty of Medicine, Universitas Sumatera Utara. The data utilized consisted of medical records from breast cancer patients who underwent comprehensive immunohistochemistry (IHC) examination between 2022 and 2024. Inclusion criteria were patients with confirmed breast cancer based on histopathological examination. Only cases diagnosed as invasive breast carcinoma were considered eligible for the study. Additionally, patients included had available immunohistochemical results for Ki67, estrogen receptor (ER), and progesterone receptor (PR) expression, ensuring complete biomarker data necessary for a correlation analysis.

Patients were excluded from the study if they had received any prior treatment for breast cancer, including chemotherapy, radiotherapy, or hormonal therapy, before tissue sampling, as such interventions could potentially alter the expression levels of Ki67, ER, or PR. Cases of non-invasive breast carcinoma, such as ductal carcinoma in situ (DCIS), were also excluded to ensure that the analysis was focused solely on invasive breast cancer. Patients diagnosed with metastatic disease at the time of initial presentation were not included, as the study aimed to examine primary tumours only.

The samples with inadequate or poor-quality tissue that precluded accurate immunohistochemical evaluation were excluded. Patients with a history of other concurrent malignancies were omitted to eliminate confounding variables that might influence biomarker expression. Male breast cancer cases were excluded to maintain a homogeneous female study population, unless otherwise specified. Furthermore, individuals with incomplete clinical or pathological data, particularly missing information regarding ER, PR, or Ki67 status, were not considered for inclusion. Depending on the study design, cases of triple-negative breast cancer (lacking ER, PR, and HER2 expression) may have also been excluded if the objective was to focus exclusively on hormone receptor-positive subtypes.

Methods

The IHC examination was conducted following the standard operating procedures (SOP) of the Anatomical Pathology Laboratory at Teaching Hospital. Estrogen receptor (ER) and progesterone receptor (PR) status was assessed using immunohistochemistry (IHC) and classified as positive or negative based on the H-score system. The H-score is a semi-quantitative method that takes into account both the percentage of positively stained tumour cells and the intensity of staining. The final H-score ranges from 0 to 300. A commonly used cut off for positivity is H-score ≥10 or ≥100, depending on the study and guidelines followed (12).

Ki67, a nuclear protein expressed during all active phases of the cell cycle (G1, S, G2, and mitosis), is a well-established marker of cellular proliferation. It is absent in quiescent (G0) cells, making it a reliable indicator of tumour cell growth. In breast cancer, Ki67 expression serves as an important prognostic and predictive biomarker, contributing to tumour grading and therapeutic decision-making.

In this study, Ki67 expression was evaluated using immunohistochemistry (IHC) on formalin-fixed, paraffin-embedded (FFPE) breast cancer tissue sections. The Ki67 antigen was detected using the MIB-1 monoclonal antibody, which is widely used for this purpose (13). Following standard IHC protocols (including deparaffinization, antigen retrieval, blocking of non-specific binding, and incubation with the primary antibody), the immune reaction was visualized using a chromogenic substrate (diaminobenzidine, DAB), resulting in a brown nuclear stain in positively labelled cells.

Ki67 expression was quantified by calculating the percentage of tumour cell nuclei showing positive staining. Only unequivocally stained tumour nuclei were included in the assessment. Evaluation was performed in areas of highest labelling, commonly referred to as “hot spots,” with at least 500–1000 tumour cells counted per case. The Ki67 labelling index was expressed as the percentage of positive nuclei out of the total number of tumour cells assessed.

Although there is no universally accepted cut off value for Ki67 expression, many studies and guidelines suggest a threshold of 20% to distinguish between low and high proliferative activity (14). In this study, the classification of Ki67 expression followed the recommendations of the International Ki67 in Breast Cancer Working Group, which provides standardized guidance for assessment and interpretation in both clinical and research settings (15).

The percentage of Ki67-positive cells, also referred to as the Ki67 proliferation index, was calculated by immunohistochemically staining tumour tissue sections and evaluating the nuclear expression of the Ki67 antigen. The assessment focused specifically on tumour cell nuclei, excluding stromal, inflammatory, or non-neoplastic epithelial cells. To determine the proliferation index, microscopic examination was performed at high magnification (typically ×400) in areas of the tumour demonstrating the most intense Ki67 staining, often referred to as “hot spots”. Within these hot spots, at least 500 to 1000 tumour cells were counted manually or using digital image analysis tools. The number of positively stained nuclei was then expressed as a percentage of the total number of tumour cells counted. In this study, the Ki67 proliferation index was categorized into two groups based on established clinical guidelines (16) to low proliferation as Ki67 index < 20%, and high proliferation as Ki67 index ≥ 20%. This cutoff was consistent with recommendations from the St. Gallen International Breast Cancer Conference (17) and the International Ki67 in Breast Cancer Working Group (18), which suggest 20% as a practical threshold for distinguishing biologically less active tumours from those with higher proliferative potential.

Statistical analysis

The association between ER/PR expression and Ki67 proliferation index was analysed by calculating the frequency distribution and receptor status percentages using the $\chi 2$ test.

RESULTS

The most common subtype was Luminal A, observed in 179 (36.3%) patients, followed by the HER2-enriched subtype with 129 (26.2%). Luminal B HER2-negative was identified in 79 (16.0%) patients, while both Luminal B HER2-positive and triple-negative breast cancer (TNBC) were each found in 53 (10.8%) patients. Out of a total of 493 patients, ER/PR negative had Ki67 >20% with 41.1% and 58.6%, respectively. Patients with ER/PR positive both had Ki67 <20% with a percentage of 36.3% (Table 1).

Table 1. Breast cancer molecular subtype frequency distribution

Subtype	No (%)
Luminal A	179 (36.3)
Luminal B Her (+)	53 (10.8)
Luminal B Her (-)	79 (16)
Her 2 Type	129 (26.2)
TNBC	53 (10.8)
Total	493

TNBC, triple negative breast cancer

The Ki67 < 20% (low proliferation index) was associated with ER/PR positive overall. Ki67 >20% (high proliferation index) showed an increased frequency in ER/PR negative. The p<0.001 also indicated a significant relationship between Ki67 and ER/PR status (Table 2).

Table 2. Result of estrogen receptor, progesterone receptor, and Ki67

Ki67 proliferation index	No (%)				p
	ER		PR		
	Positive	Negative	Positive	Negative	
< 20%	179 (36.3)	0	179 (36.3)	0	
> 20%	110 (22.3)	204 (41.4)	25 (5.1)	289 (58.6)	
Total	289 (58.6)	204 (41.4)	204 (41.4)	289 (58.6)	<0.001

ER, estrogen receptor; PR, progesterone receptor

DISCUSSION

Our findings establish the frequency distribution of different breast cancer subtypes prior to analysing the relationship between ER/PR status and Ki67. The results of the analysis are consistent with a previous study, which showed an inverse relationship between ER and PR with Ki67 (5). Ki67 is expressed during the cell cycle and is bound when the cell divides. Therefore, Ki67 can be used as an indicator of the rate of development of both normal and malignant cells. This protein affects the speed of cell division, which can then impact the prognosis of breast cancer (12).

ER/PR status has an inverse correlation with clinical stage status. Thus, the expression of ER and PR can be used as prognostic factors to select therapy regimens (13). Patients with ER/PR positive are recommended for hormonal therapy, while those with ER/PR negative are recommended for neoadjuvant chemotherapy therapy. However, the expression of ER and PR alone is not sufficient to accurately determine a clinical outcome (14). Additional factors such as Ki67 and cancer cell proliferation index can provide a more comprehensive picture of the prognosis and treatment response for optimal care.

Although gene expression profiling studies are increasingly being developed to assess the prognostic risk of breast cancer, tumour classification using the IHC system, including Ki-67 assessment, remains of significant value (15). IHC system is more efficient, affordable, and can be routinely used in clinical practice. Factors such as tumour size, nodal involvement, and tumour grade still play a role as prognostic indicators (16), but they now need to be complemented with new biological parameters.

Ki-67 has proven to be an independent predictor of survival (17). In Luminal B breast cancer with negative nodes, Ki-67 assessment becomes an important prognostic factor, while PR expression does not show a similar role (18). Standardization of accurate Ki-67 assessment will improve the estimation of breast cancer recurrence risk (19). Every breast cancer patient should undergo testing for four biomarkers, namely ER, PR, HER2neu, and Ki67 (20). Specifically, high Ki67 expression in cases with ER and PR negative is associated with a worse prognosis (21). However, breast cancer with high Ki67 expression tends to respond better to chemotherapy (22).

This study has several limitations that should be acknowledged. First, the data were derived from a single centre in Medan, which may limit the generalizability of the findings to broader populations or other healthcare settings. The single-centre design could introduce institutional bias in patient selection, pathological assessment, and treatment protocols. Secondly, Ki67 testing has not yet been uniformly implemented across all medical centres in Medan or throughout the country. Variations in testing availability, IHC protocols, interpretation methods, and cutoff values may affect the reproducibility and standardization of Ki67 assessment, which remains a challenge in many regions. Additionally, this study did not include long-term follow-up data, limiting its ability to directly correlate Ki67 expression with patient outcomes such as overall or disease-free survival. Future multicentre studies with standardized Ki67 evaluation protocols and prospective follow-up are recommended to validate these findings.

In conclusion, ER/PR status showed an inverse relationship with Ki67. High Ki67 can be associated with a higher frequency of ER/PR negativity and vice versa. Ki67 expression can be determined by the frequency of ER/PR status, but both have different functions in the treatment of breast cancer patients. ER and PR expression play a role in determining treatment options, while Ki67 expression functions to assess prognosis. The originality of this study lies in its focus on the correlation between Ki67 expression and hormone receptor (ER and PR) status in breast cancer patients within a single-centre population in Medan, Indonesia - a setting where standardized Ki67 assessment is not yet routinely implemented. While numerous international studies have investigated Ki67 as a prognostic and predictive marker, regional data from Indonesia remain limited. This study contributes to novel insights by providing local evidence of Ki67 distribution patterns and their relationship with ER and PR status, potentially reflecting unique biological or demographic characteristics of the local patient population. Furthermore, it highlights the pressing need for broader implementation and standardization of Ki67 testing in routine diagnostic workflows within the country. By contextualizing findings within our healthcare setting, this study adds meaningful data to the global understanding of breast cancer biomarkers in diverse clinical environments.

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TRANSPARENCY DECLARATION

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Author contributions statement

Conceptualization, supervision, and validation: DH and ETP; Methodology, formal analysis, and data curation: MB, MAA, KSA; Writing original draft, analysis, and visualization: KHA and MAA; Review draft and project administration: DH and KHA.

REFERENCES

1. Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, et al. Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. CA Cancer J Clin [Internet]. 2021 May;71(3):209–49.
2. Anggorowati L. Faktor Risiko Kanker Payudara Wanita (Risk Factors for Female Breast Cancer) [in Bahasa Indonesia]. KESMAS - J Kesehat Masy. 2013;8(2):121–6.
3. Alfina AID, Sandhika W, Suprabawati DGA. Nuclear size and pleiomorphism of breast cancer cells at high and low Ki-67 proliferation index. World J Adv Res Rev 2024; 21(1):1132–5.
4. Hu X, Chen W, Li F, Ren P, Wu H, Zhang C, et al. Expression changes of ER, PR, HER2, and Ki-67 in primary and metastatic breast cancer and its clinical significance. Front Oncol 2023; 13:1–9.
5. Sheikhpour R, Poorhosseini F. Relation between estrogen and progesterone receptor status with p53, ki67 and her-2 markers in patients with breast cancer. Iran J Blood Cancer 2016; 8(4):93–7.
6. Prakash DM, Shivakumar S, Bapsy PP. A retrospective study of immunohistochemical detection of Ki67 and correlation with ER, PR, and HER2-neu status in invasive breast cancer. J Clin Oncol 2024; 30(15_suppl):e21132–e21132.
7. Patel R, Hovstadius M, Kier MW, Moshier EL, Zimmerman BS, Cascetta K, et al. Correlation of the Ki67 Working Group prognostic risk categories with the Oncotype DX Recurrence Score in early breast cancer. Cancer 2022; 128(20):3602–9.
8. Soliman NA, Yussif SM. Ki-67 as a prognostic marker according to breast cancer molecular subtype. Cancer Biol Med 2016; 13(4):496.
9. Strimbu K, Tavel JA. What are Biomarkers? Curr Opin HIV AIDS. 2010; 5(6):463.
10. Reddy DP, Devi S DM. Correlation of ER, PR, Her2neu and Ki67 with other Prognostic factors in Breast Carcinoma. Trop J Pathol Microbiol 2020; 6(5):349–61.
11. Rakha, E. A., Reis-Filho, J. S., Baehner, F., Dabbs, D. J., Decker, T., Eusebi, V., Fox, S. B., Ichihara, S., Jacquemier, J., Lakhani, S. R., Palacios, J., Richardson, A. L., Schnitt, S. J., Schmitt, F. C., Tan, P. H., Tse, G. M., Badve, S., & Ellis, I. O. (2010). Breast cancer prognostic classification in the molecular era: the role of histological grade. *Breast cancer research : BCR*, *12*(4), 207. https://doi.org/10.1186/bcr2607
12. de Azambuja E, Cardoso F, de Castro G Jr, Colozza M, Mano MS, Durbecq V, et al. Ki-67 as prognostic marker in early breast cancer: a meta-analysis of published studies involving 12,155 patients. Br J Cancer 2007; 96(10):1504–13. doi:10.1038/sj.bjc.6603756
13. Sato, N., Tsujimoto, M., Nakatsuji, M., Tsuji, H., Sugama, Y., Shimazu, K., Shimoda, M., & Ishihara, H. (2024). Flow cytometric analysis for Ki67 assessment in formalin-fixed paraffin-embedded breast cancer tissue. BMC biology, 22(1), 181. https://doi.org/10.1186/s12915-024-01980-4
14. Bustreo, S., Osella-Abate, S., Cassoni, P., Donadio, M., Airoldi, M., Pedani, F., Papotti, M., Sapino, A., & Castellano, I. (2016). Optimal Ki67 cut-off for luminal breast cancer prognostic evaluation: a large case series study with a long-term follow-up. Breast cancer research and treatment, 157(2), 363–371. https://doi.org/10.1007/s10549-016-3817-9
15. Inwald, E. C., Klinkhammer-Schalke, M., Hofstädter, F., Zeman, F., Koller, M., Gerstenhauer, M., & Ortmann, O. (2013). Ki-67 is a prognostic parameter in breast cancer patients: results of a large population-based cohort of a cancer registry. *Breast cancer research and treatment*, *139*(2), 539–552. https://doi.org/10.1007/s10549-013-2560-8
16. Dokcu, Ş., Ali-Çaparlar, M., Çetindağ, Ö., Hakseven, M., & Eroğlu, A. (2023). Valor pronóstico del índice de proliferación KI-67 en cánceres de mama luminales. [(Prognostic value of KI-67 proliferation index in luminal breast cancers.) [in Spanish] *Cirugia y cirujanos*, *91*(1), 1–8. https://doi.org/10.24875/CIRU.22000043
17. Goldhirsch, A., Winer, E. P., Coates, A. S., Gelber, R. D., Piccart-Gebhart, M., Thürlimann, B., Senn, H. J., & Panel members (2013). Personalizing the treatment of women with early breast cancer: highlights of the St Gallen International Expert Consensus on the Primary Therapy of Early Breast Cancer 2013. *Annals of oncology : official journal of the European Society for Medical Oncology*, *24*(9), 2206–2223. https://doi.org/10.1093/annonc/mdt303
18. Dowsett, M., Nielsen, T. O., A'Hern, R., Bartlett, J., Coombes, R. C., Cuzick, J., Ellis, M., Henry, N. L., Hugh, J. C., Lively, T., McShane, L., Paik, S., Penault-Llorca, F., Prudkin, L., Regan, M., Salter, J., Sotiriou, C., Smith, I. E., Viale, G., Zujewski, J. A., … International Ki-67 in Breast Cancer Working Group (2011). Assessment of Ki67 in breast cancer: recommendations from the International Ki67 in Breast Cancer working group. *Journal of the National Cancer Institute*, *103*(22), 1656–1664. https://doi.org/10.1093/jnci/djr393
19. Yerushalmi R, Woods R, Ravdin PM, Hayes MM, Gelmon KA. Ki67 in breast cancer: prognostic and predictive potential. Lancet Oncol 2010; 11(2):174–83.
20. Nurcahyanti O, Kusmardi K. Imunohistokimia pada Kanker Payudara : Teknik , Aplikasi , dan Implikasinya dalam Diagnostik dan Terapi. (Immunohistochemistry Pathology Pratista in Breast Cancer: Techniques, Applications, and Implications in Diagnostics and Therapy) [in Bahasa Indonesia] Pratista Patol 2024; 9(2):185–97.

21. Wen Z, Luo D, Wang S, Rong R, Evers BM, Jia L, et al. Deep Learning-Based H-Score Quantification of Immunohistochemistry-Stained Images. 2024; Mod Pathol 37(2);100398. https://doi.org/10.1016/j.modpat.2023.100398

22. Pérez-López ME, García-Gómez J, Alves MT, Paradela A, García-Mata J, García-Caballero T. Ki-67 is a prognostic marker for hormone receptor positive tumors. Clin Transl Oncol 2016; 18(10):996.