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Association between Ki67 Index and Clinicopathological Parameters in Breast Cancer Patients in Bali

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ABSTRACT

Aim: To assess the association between Ki67 index and various clinicopathological parameters of breast cancer patients in Bali. **Methods:** This was a cross-sectional study of breast cancer patients at Prof. Dr. I. G. N. G Ngoerah Hospital, a referral hospital in Bali, from 1 January 2021 to 31 December 2021. Clinicopathology data was taken from SIMARS and archives in the Anatomical Pathology Laboratory. Ki67 index was assessed by immunohistochemical examination, categorized into low (Ki67 index <20%) and high (Ki67 index≥20%). Association between Ki67 index with clinicopathological parameters was analysis with X^2 test with significance level determined at α=0.05. **Results:** There were 75 cases of breast cancer patients with a mean age was 50.74 ± 10.05 years old. Low Ki67 index was found in 9 (12%) cases, while high Ki67 index in 66 (88%) cases. There was a significant association between Ki67 index and histological grade of the tumor (p=0.007), nodal status (p=0.014), and molecular subtype of tumor (p=0.007). There was no significant association between Ki67 index and the patient's age (p=0.268), histological subtype (p=0.086), and T stage (p=0.511). **Conclusion:** Ki67 index has significant association with histological grade of the tumor, nodal status, and the molecular type of the tumor in breast carcinoma patients in Bali.

Keywords: breast carcinoma, Ki67, immunohistochemistry, clinicopathology.

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INTRODUCTION

Currently, breast cancer is the most common malignancy in women worldwide, and its incidence is increasing.[1] Breast carcinoma is a heterogeneous tumor, both in clinical course of disease, microscopic appearance of the tumor, and molecularly. The current era also leads to a therapeutic approach to breast cancer patients that is personalized (personalized treatment). [2] In addition, therapy for breast cancer patients is highly developed, both at the research level and several new drugs have been applied to patient management.

Various clinicopathological characteristics have been used to predict prognosis of breast cancer patients. A study by Nguyen et al (2020) showed that patient age, tumor histology grade, tumor size and nodal status were independently related to overall survival and recurrence free survival in breast cancer patients.[3]

Ki67 is a nuclear protein that is expressed at most stages of the cell cycle.[2] Ki67 index is a marker of proliferation that is frequently assessed in many malignancies. In breast carcinoma, the purpose for assessing Ki67 index includes prognosis, prediction of response to or resistance to chemotherapy or endocrine therapy, estimation of residual risk in patients on standard therapy, and biomarkers of therapy efficacy.[4–6] The

purpose of this study was to determine the relationship between Ki67 index and various clinicopathological parameters of breast cancer patients in Bali.

METHODS

This was a cross sectional study using breast cancer patient who underwent radical mastectomy and axillary lymph node dissection in Prof. Dr. I. G. N. G Ngoerah Hospital, a province referral hospital in Bali, in the year 2021, and Formalin fixed paraffin embedded (FFPE) tissue from tumor biopsy or mastectomy before chemotherapy was available. Clinicopathology data (patient's age, histological subtypes, histological grade, T stage, nodal status, and molecular subtypes) were obtained from SIMARS and data in the Pathology examination archives at the Anatomical Pathology Laboratory of Prof. Dr. I. G. N. G Ngoerah Denpasar.

Ki67 was evaluated by immunohistochemistry. FFPE tissue was retrieved from archives at the Anatomical Pathology Laboratory of Prof. Dr. I. G. N. G Ngoerah in the year 2021. The staining was done by Bondmax autostainer. Monoclonal antibody anti-Ki67 PA0118 Bond RTU Primary Antibody (Leica Biosystems) was used for detection. Heat antigen retrieval was done with EDTA at pH 9, in 100^0C, for 20 minutes.

Evaluation of Ki67 index was carried out using an Olympus CX21 microscope connected to an Optilab microphoto and equipped with Image Raster software. Interpretation was carried out in 3 fields of view with 400x magnification or until 500 tumor cells are identified. Cut off value to categorized high and low Ki67 index was determined at 20%.[7]

This study obtained ethical approval from Research Ethical Commission of Faculty of Medicine, Udayana University with ethical approval number: l720lWl4.2.2.ylI.l4lLT12022. Research permission from Prof. Dr. I. G. N. G Hospital was also obtained with permission number: LB.02.01/VIV.2.2.2/34534/2022.

Descriptive analysis which includes the clinicopathologic characteristics of the patients. The relationship between the Ki67 index and various clinicopathological parameters was tested with X^2 test. The significance test was determined at α=0.05.

RESULTS

During the year 2021 at the Anatomical Pathology Laboratory of Prof. Dr. I. G. N. G Ngoerah collected 75 cases of breast carcinoma which had complete clinicopathological data and FFPE from biopsy or mastectomy prior chemotherapy was available. The mean age of the patients was 50.74 ± 10.05 years, with an age range of 22-74 years (**Table 1**).

Cases of breast carcinoma with a low Ki67 index were found in 9 (12%) cases, while a high Ki67 index was found in 66 (88%) cases. The relationship between Ki67 expression and various clinicopathological parameters is shown in the **Table 2**.

There was a significant relationship between the Ki67 index and the histological grade of the tumor (p=0.007), nodal status (p=0.014), and the molecular type of tumor (p=0.007). There was no significant relationship between the Ki67 index and the patient's age (p=0.268), histological type of tumor (p=0.086), and stage T (p=0.511).

Table 1. Distribution of clinicopathological data on breast cancer patients at Prof. Dr. I.G.N.G Ngoerah in the year 2021.

Parameter	**n=75**	**%**
Age		
≤50 year old	38	50.7
>50 year old	37	49.3
Histological type		
IBC, no special type	55	73.3
IBC, special type	20	26.7
Histological grade		
Grade 1-2	35	46.7
Grade 3	40	53.3
T stage		
T1-T2	34	45.3
T3-T4	41	54.7
Nodal status		
Negative	30	40.0
Positive	45	60.0
Molecular subtype		
ER/PR+, HER2-	28	37.3
ER/PR+, HER2+	15	20.0
ER/PR-, HER2+	14	18.7
ER/PR-/HER2-	18	24.0

Table 2. The relationship between Ki67 expression and various clinicopathological parameters in breast cancer patients at Prof. Dr. I.G.N.G Ngoerah in the year 2021.

Parameter	**Ki67 index**		**p**
	Low (<20%) n=9	**High (≥20%) n=66**	
Age			
≤50 year old	3 (7.9%)	35 (92.1%)	0.268
>50 year old	6 (16.2%)	31 (83.8%)	
Histological type			
IBC, no special type	5 (9.1%)	50 (90.9%)	0.199
IBC, special type	4 (20.0%)	16 (80.0%)	
Histological grade			
Grade 1-2	8 (22.9%)	27 (77.1%)	0.007
Grade 3	1 (2.5%)	39 (97.5%)	
T stage			
T1-T2	5 (14.7%)	29 (85.3%)	0.511
T3-T4	4 (9.8%)	37 (90.2%)	
Nodal status			
Negative	7 (23.3%)	23 (76.75%)	0.014
Positive	2 (4.4%)	43 (95.6%)	
Molecular subtype			
ER/PR+, HER2-	8 (28.6%)	20 (71.4%)	0.007
ER/PR+, HER2+	1 (6.7%)	14 (93.3%)	
ER/PR-, HER2+	0 (0.0%)	14 (100%)	
ER/PR-/HER2-	0 (0.0%)	18 (100%)	

DISCUSSION

Continuous cell proliferation is one of the hallmarks of cancer. The rate of cell proliferation can be evaluated by several methods including thymidine labeling index, S-phase fraction as determined by flow-

cytometry, and Ki67 immunohistochemical examination.[8] Ki67 is a nuclear protein that is expressed in all phase of the cell cycle. The Ki67 index is a marker of proliferation that is frequently assessed in many malignancies. The Ki67 assessment has a prognostic and predictive role in breast cancer patients.[4,5] In this study, there was a significant relationship between the Ki67 index and the histological grade of the tumor, nodal status, and molecular subtype.

High Ki67 index is found more in IBC cases with high histologic grade (grade 3), consistent with other studies. Study by Hashmi et al. found that Ki67 index is significantly related to the histological grade of the tumor, but not to other clinicopathological parameters.[8] Study by Ragab et al. found that Ki67 index increases with increasing tumor size and grade.[9] Histologic grading system used in breast carcinoma is Nottingham Grading system that evaluate 3 parameters which are: nuclear pleomorphia, tubular formation, mitotic index.[10] Ki67 is a proliferation index, as well as mitotic index.

High Ki67 index was found more in IBC cases with axillary nodal metastasis. This finding is consistent with Yin et al. which found high Ki67 index positively correlated with the occurrence of nodal metastases.[11] Previous study by Putra et al. in Sanglah General Hospital, Bali also reported that high Ki67 expression was associated with staging in breast cancer patient.[12] Nodal metastasis is the most important predictor of distant metastases risk, recurrence & survival in IBC.[13] Ki67 status is an independent prognostic factor for metastatic free survival (MFS) in patients with 1-3 positive axillary nodes.[14]

Ki67 index had significant correlation with molecular subtypes. Most of HER2 positive breast cancer and TNBC subtypes showed high Ki67 index. Meanwhile, the proportion of low Ki67 index was more often in IBC ER/PR+ HER2- than other subtypes. Many studies found consistent results.[15] Study by Al-Zawi et al. showed high Ki67 levels more frequent in Luminal B, HER2-enriched & TNBC.[16] Study by Ragab et al found Ki67 index is lower in ER/PR+ than ER/PR-. High Ki67 index more often on HER+.[9] Molecular subtype of tumor, apart from being the basis for determining individual therapy, is also important for prognosis.[17]

Immunohistochemistry for Ki67 is a simple procedure and nowadays this testing is available in many pathological laboratory. Valid evaluation of Ki67 index is important. Optimal pre-analytic process, standardized laboratory procedures and pathological evaluation (interpretation, scoring and cut off value) is important.[6] Optimal pre-analytic process is responsibility of surgical team. In the era of digital pathology and artificial intelligence, the second issue can be minimized.

CONCLUSION

There was a significant association between the Ki67 index and the histological grade of the tumor, nodal status, and the molecular type of the tumor in breast carcinoma patients. There was no significant relationship between the Ki67 index and the patient's age, tumor histological type, and tumor T stage. The application of Ki67 together with other clinicopathological factors can improve the prediction of the prognosis of IBC patients. Standardized evaluation of Ki67 index is important to get valid measurement.

DECLARATIONS

Authors declare no competing interest in this study.

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