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The Association Between Ki-67 and P53 Expression with Histopathological Grading in Breast Cancer: A Cross-Sectional Study

John Pieter Jr[1,2*], William Hamdani[1,2], Berti Julian Nelwan[3], Muhammad Faruk[4]

[1]Division of Oncology, Department of Surgery, Faculty of Medicine, Hasanuddin University, Makassar, Indonesia
[2]Division of Oncology, Department of Surgery, Siloam Hospital, Makassar, Indonesia
[3]Department of Pathology Anatomy, Faculty of Medicine, Hasanuddin University, Makassar, Indonesia
[4]Department of Surgery, Faculty of Medicine, Hasanuddin University, Makassar, Indonesia

***Corresponding author:**
John Pieter Jr, MD
Division of Oncology
Department of Surgery
Faculty of Medicine
Hasanuddin University, Makassar
Jalan Perintis Kemerdekaan KM 11
Makassar, South Sulawesi, 90245
Indonesia
Telephone: +62411585984
Fax: +62411585984
E-mail: john_pieterjr@yahoo.com

ABSTRACT

Background: Rapid developments in molecular biology in recent years have made possible the discovery of several new prognostic factors in addition to the previously known ones. Changes in proto-oncogenes and suppressor genes play an important role in the pathogenesis of cancer. The proto-oncogene Ki-67 and the suppressor gene P53 are prognostic and predictive markers for the level of proliferation. This study aims to determine the association between Ki-67 and P53 in patients with breast cancer.
Methods: This cross-sectional study examined 40 cases analyzing the expression of Ki-67 and P53 by immunohistochemistry assay. The association of Ki-67 and P53 expression with histopathological grading was tested with Spearman's rho test.
Results: Ki-67 was expressed in 32 of 40 patients with breast cancer (80%), mostly with a high histopathological grade (65%). P53 was expressed in 33 of 40 patients with breast cancer (82.5%), mostly with a high histopathological grade (65%). Associations were evaluated between the expression of Ki-67 and histopathological grade ($p \leq 0.001$), P53 and histopathological grade ($p \leq 0.001$), and P53 expression with Ki-67 expression ($p = 0.059$).
Conclusion: The associations observed demonstrate the relationship between Ki-67 and P53 expression with histopathological grading of breast cancer.
Key words: Ki-67, P53, breast cancer, immunohistochemistry, histopathological grade

INTRODUCTION

Rapid developments in molecular biology in recent years have made possible the discovery of several new prognostic factors in addition to the previously known prognostic factors (1,2). Many oncological studies have sought to find methods of predicting the course of breast cancer (BC) to enable quick, precise, and optimal selection of therapies (1,3), thus improving the chances of prognoses of disease-free survival for BC patients (4).

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Changes in proto-oncogenes and suppressor genes play an important role in the pathogenesis of cancer. In normal cells, the expression of proto-oncogenes is required for normal cell development and growth and does not cause malignancy because its activity is precisely controlled (5). Mutations from proto-oncogenes to oncogenes can occur through structural changes in genes, chromosomal translocations, gene amplification, or mutations in various elements that normally function to control the expression of the relevant gene (5,6). Proto-oncogenic mutations are relatively common in actively proliferating cells, but malignant changes can be inhibited through the expression of various suppressor genes (tumor suppressor genes or anti-oncogenes) that play a role in cell cycle arrest or apoptosis. If genes that play a role in surveillance are disrupted due to mutations or deletions, the affected cells become susceptible to malignant transformation (5,6).

Many markers are used to assess the aggressiveness of cancer cells through calculation of their rate of proliferation. The Ki-67 gene and the P53 gene are prognostic and predictive markers of the level of proliferation (2,7–10). A wide study evaluating the relationship between the proto-oncogene Ki-67 and the suppressor gene P53 has not previously been conducted in Indonesia; therefore, we are interested in examining the relationship between the two genes in patients with BC.

METHODS

This research was conducted using a cross-sectional analysis method at Dr. Wahidin Sudirohusodo Hospital, Makassar, Indonesia, from September 2020 to February 2021, and received approval from the Health Research Ethics Commission of the Faculty of Medicine, Hasanuddin University (number: UH20120708).

We carried out the immunohistochemistry (IHC) examination using the monoclonal antibodies Ki-67 and P53 at the Anatomy Pathology Laboratory of the Faculty of Medicine, Hasanuddin University, Makassar, Indonesia. The histopathological grade was divided into 3 groups: low, moderate, and high (based on the Modified Scarff–Bloom–Richardson grading method).

Population and sample

The study population consisted of all patients that were diagnosed with BC through clinical and pathological examination. The research subjects were all patients with BC who were treated at our institution. The inclusion criteria for the study were: BC patients who had never received treatment before; BC patients who were not affected by other malignancies; and BC patients who were willing to participate in the study. The exclusion criteria were: patients with residual BC; BC patients who were temporarily pregnant, breast-feeding or undergoing hormone therapy; and BC patients whose tissue samples were unrepresentative.

We obtained samples from all patients with BC (based on clinical and histopathological examination). Tissue samples were from a mastectomy or biopsy and pre-existing tissue paraffin blocks.

Sample preparations

Surgical tissue preparations from samples diagnosed with invasive ductal BC were then sent to the Pathological Anatomy Laboratory, Faculty of Medicine, Hasanuddin University, Makassar. The samples were stained with hematoxylin-eosin staining, and we recorded the names, ages, and numbers of the anatomical pathology preparations. We then reassessed the histopathological score.

Immunohistochemistry (IHC)

The sample was then stained for immunohistochemistry purposes using the indirect immunoenzyme technique and using a labeled streptavidin complex. The primary antibody test was the Dako monoclonal antibody test (mouse monoclonal anti-human Ki-67 Antigen Clone MIB-1, Catalog No. GA626 and mouse monoclonal anti-human P53 Antigen Clone DO-1, Catalog No. sc-126), used according to the manufacturer's instructions (11). The results were obtained by examining the sample under an optical microscope at magnifications of 10x and 40x.

Interpretation of the IHC results

The level of Ki-67 is considered negative if the staining in 100 visual fields shows an expression in < 5% of the fields, low if the staining in 100 visual fields shows an expression in 5-20% of the fields, moderate when Ki-67 expression is 20–50%, and high when Ki-67 expression is >50%.

We observed the level of P53 expression from the percentage of cell groups that showed positive P53 staining. The expression of P53 is positive if 25% of the nuclei of the tumor cells are brown. Preparations for ovarian cancer were used as positive controls, as they are known to have a positive expression of P53. The

negative controls used preparations for BC without the use of primary antibodies.

Data analysis

We processed all data, and Spearman's rho statistical test was performed. The results are displayed in the form of tables. Data analysis was performed using SPSS version 22 (IBM Corp. Released 2013. IBM SPSS Statistics for Windows, Version 22.0. Armonk, NY: IBM Corp.). Test results were deemed statistically significant if the p value of the test was <0.05.

RESULTS

The pathological anatomical descriptions of Ki-67 and P53 expression in this study using IHC examination are shown in *figs. 1* and *2*, respectively. We observed the level of P53 expression from the percentage of cell groups showing positive P53 staining.

The characteristics of this study are described in detail in *table 1*. The most populated age distribution was ≤ 50 years old (72.5%). The most common histopathological grade was "high," in 27 people (67.5%). We found a positive expression of Ki-67 in 32 cases (80%) of BC patients. The most common expression of Ki-67 was "Positive 3" (13 cases; 32.5%). Positive expression of P53 was found in 33 cases (82.5%), with the most common expression of P53 occurring at level "Positive 2" in 23 samples (57.5%).

Table 1 - Sample characteristics

Characteristics	n	(%)
Age (years)		
≤ 50	29	72.5
> 50	11	27.5
Tumor grading		
Low grade	1	2.5
Moderate grade	12	30.0
High grade	27	67.5
Ki-67 expression		
Negative	8	20.0
Low	9	22.5
Moderate	10	25.0
High	13	32.5
P53 expression		
Negative	7	17.5
Positive 1	10	25.0
Positive 2	23	57.5

Analysis of the relationship between the expression of Ki-67 and tumor grading

The distribution of the expression of Ki-67 with the histopathological grade of all the samples in this study is presented in *table 2*. The qualification levels of the expression of Ki-67 were divided according to Group 1 (expression with degrees +1, +2, and +3) and Group 2

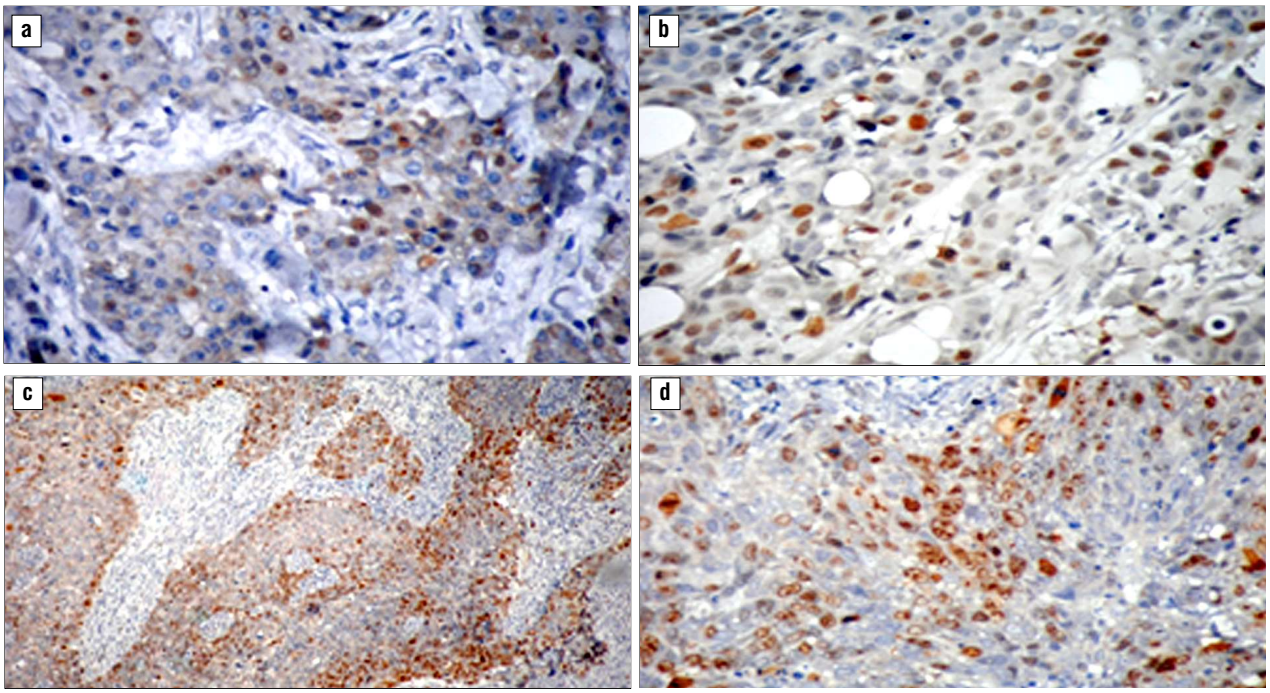

Figure 1 - (a) Positive immunostaining for Ki-67 ≤ 20% (low), x400. (b) Positive immunostaining for Ki-67 20–50% (moderate), x400. (c) Positive immunostaining for Ki-67 >50% (high), x100. (d) Positive immunostaining for Ki-67 >50% (high), x400

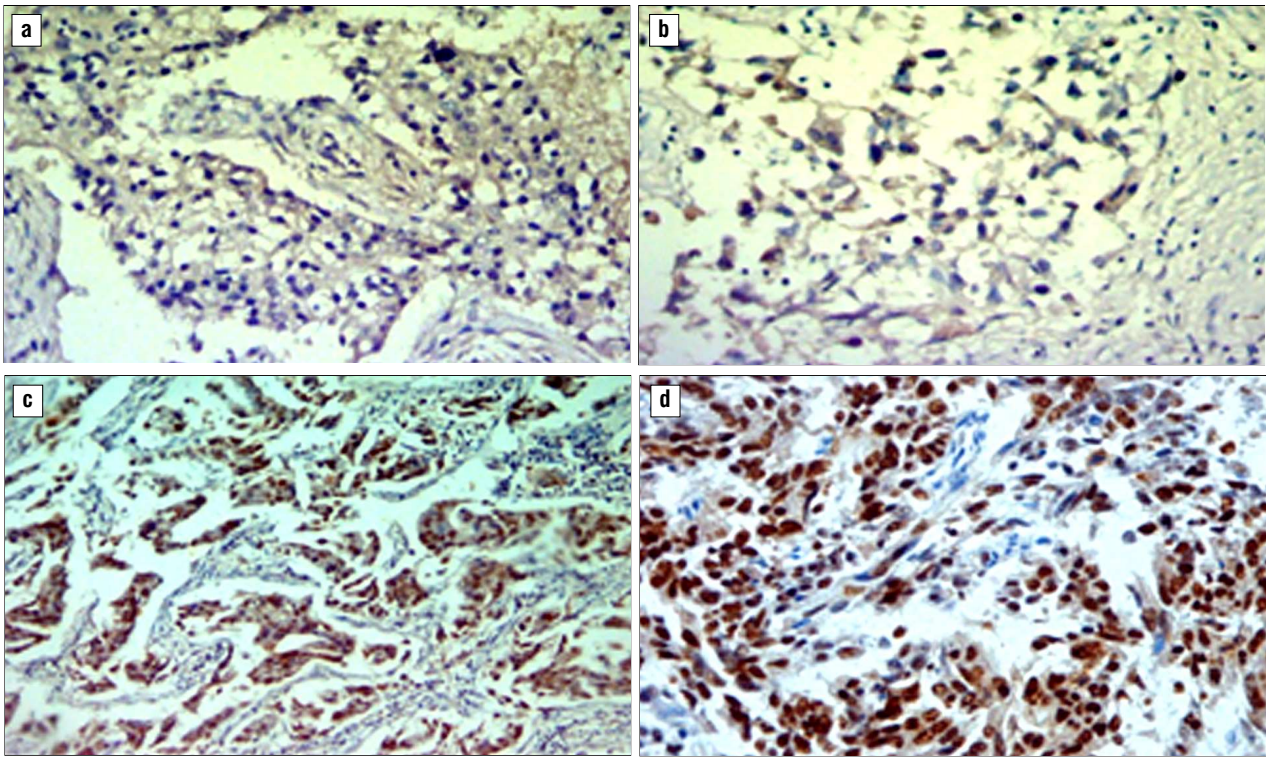

Figure 2 - (a) Negative immunostaining for P53, x200. (b) Negative immunostaining for P53, x400. (c) Positive immunostaining for P53, x200. (d) Positive immunostaining for P53, x400

(negative expression). The histopathological grading has been divided into 3 groups: low grading, middle grading, and high grading. The highest expression of Ki-67 was in Group 1 (positive expression) with a high histopathological grade of 26 samples (65%), and the least common expression of Ki-67 was in Group 1 (positive expression) with low histopathological grade in 0 samples (0%).

Based on Spearman's rho correlation test, the significance value of $p \leq 0.001$ was obtained in the histopathological ranking relation test with the qualification level of Ki-67 expression of this study sample. The value is less than the threshold defining statistical significance, meaning there is a strong relationship between the qualification level of Ki-67 expression and the histopathological grade. The value of the correlation coefficient is 0.604 with a positive direction.

Analysis of the relationship between the expression of P53 and tumor grading

The distribution of P53 expression with the histopathological grade of all study samples is shown in *table 3*. Qualifying levels of P53 expression were divided according to Group 1 (expression with positive degrees + 1 and +2) and Group 2 (negative expression). The histopathological grade was divided into 3 groups, namely: Group 1 (low grade), Group 2 (medium grade),

Table 2 - Distribution of Ki-67 expression with tumor grading

Tumor grading	Ki-67 expression			
	Group 1 (+)		Group 2 (-)	
	n	%	n	%
Low grade	0	0	1	2.5
Moderate grade	6	15.0	6	15.0
High grade	26	65.0	1	2.5
TOTAL	32	80.0	8	20.0

Note: Expression Ki-67: Group 1 = expression of degrees +1, +2, and +3, and Group 2 = negative.

Table 3 - Distribution of P53 expression with tumor grading

Tumor grading	P53 expression			
	Group 1 (+)		Group 2 (-)	
	n	%	n	%
Low grade	1	2.5	0	0.0
Moderate grade	6	15.0	6	15.0
High grade	26	65.0	1	2.5
TOTAL	33	82.0	7	17.5

Note: Expression P53: Group 1 = expression of degrees +1, +2, and +3, and Group 2 = negative.

and Group 3 (high grade). The highest expression of P53 was in Group 1 (positive expression) with a "high" histopathological grade (26 samples; 65%), and the lowest was in Group 2 (negative expression) with a low histopathological grade (0 samples; 0%).

Based on Spearman's rho correlation test, a significance value of $p \leq 0.001$ was obtained in the histopathological ranking relationship test with the skill level of P53 expression of this study sample. The value is less than the threshold defining statistical significance, meaning that there is a strong relationship between the skill level of P53 expression and the histopathological grade. The value of the correlation coefficient is 0.499 with a positive direction.

Analysis of the relationship between the P53 and Ki-67 expressions

The distribution of P53 expression with Ki-67 expression from all samples in this study is shown in *table 4*. Qualifying levels of P53 expression were divided according to Group 1 (expression with degrees +1 and +2) and group 2 (expression with negative degrees). At the same time, the qualification level of Ki-67 expression was divided into 2 groups, namely: Group 1 (expression with degrees +1, +2, and +3) and Group 2 (expression with negative degree). The highest simultaneous expression of P53 and Ki-67 was in the positive expression of 29 samples (72.5%), and the lowest was in the negative expression of P53 and the positive expression of Ki-67 in 3 samples (7.5%).

Based on Spearman's rho correlation test, we obtained a significance value of $p = 0.059$ in testing the relationship between the skill level of the P53 expression and the Ki-67 expression of this research sample. The value is greater than the threshold defining statistical significance, meaning that there is no significant relationship between the skill level of P53 expression and Ki-67 expression. The value of the correlation coefficient is also low, amounting to 0.302 in the negative direction.

Table 4 - Distribution of P53 expression with Ki-67 Expression

P53 expression	Ki-67 Expression			
	Group 1 (+)		Group 2 (-)	
	n	%	n	%
Group 1 (+)	29	72.5	4	10.0
Group 1 (+)	3	7.5	4	10.0
TOTAL	32	80.0	8	20.0

Note: Ki-67 expression: Group 1 = expression of degrees +1, +2, and +3, Group 2 = negative; P53 expression: Group 1 = expression of degrees +1, +2, and +3, and Group 2 = negative.

DISCUSSION

BC genes can act as proto-oncogenes that regulate the progression of cancer cell growth and as tumor suppressors (5). The Ki-67 gene is a proto-oncogene that can provide insight into the rapid rate of cell proliferation. In contrast, the P53 gene is a gene that inhibits cell growth (12).

There is evidence to suggest the development and progression of cancer does not occur until mutations in genes accumulate (13). Activation of proto-oncogenes to oncogenes due to genetic mutations and inactivation of suppressor genes occurs because there is an expression or a protein that can bind the production of the suppressor gene (12,14). Mutations in genes or chromosomes can take the form of translocations, deletions, additions, or inactivation or amplification of genes (13).

The overexpression of genes that regulate the high rate of cell proliferation in BC will indicate the level of progression and aggressiveness (3). Clinical manifestations are characterized by progressive cancer growth leading to metastasis and poor overall survival (3,15). Expression of the Ki-67 and P53 gene can be observed by IHC examination (10).

Based on the data in *table 1*, we found that the group with the largest number of people suffering from BC was the ≤ 50-year-old group (29 people; 72.5%). This result contrasts with the results of numerous studies around the world which indicate that the peak incidence of BC is at an average age of over 50 years. The American Cancer Society reported 231,840 new BC cases in women within the United States in 2015, and the most vulnerable group (59,990 cases) was those aged 60–69 years old (4,16). However, the results of this study are similar to the findings of Indra et al. (11), who reported that the average age of breast cancer patients in Indonesia is between 50 and 60 years old (11).

Our study found a positive expression of Ki-67 in 80% of the cases (32 cases) of BC in this study. The highest expression of Ki-67 was "Positive 3" for 32.5% (13 cases). At the same time, positive expression of P53 was found in 82.5% of cases (33 cases), with the highest expression of P53 being "Positive 2" occurring in 57.5% of cases (23 cases). The results of expression of the Ki-67 and P53 genes in this study were slightly different from the results obtained by Lebe et al. (32), who found Ki-67 expression in 62.5% of cases and P53 expression in 75% (24 cases out of 32 cases).

Based on *table 2*, we found the distribution of BC patients by histopathologic grade group to be highest in the "high" grade (27 samples; 67.5%). The remainder

were in the “moderate” grade (12 samples; 30%) and “low” grade (1 sample; 2.5%). At the same time, the highest level of data from the Ki-67 expression group was 32 samples (80.0%). The correlation test results with Spearman's rho test obtained a very high significance value (p = < 0.001), with a moderate degree of positive correlation coefficient of 0.604. This shows a relationship between the level of expression of Ki-67 and the level of histopathological grade. The stronger the Ki-67 expression, the higher the level of histopathological grade among sufferers in BC. This study’s findings are similar to other studies that reported that high Ki-67 expression was positively associated with a high grade; this was not associated with tumor size and regional lymph node invasion (17-19).

The analysis of mutant P53 expression in this study used immunohistochemical techniques to observe the accumulation of mutant protein in tumor tissue encoded by the P53 gene. This technique was used because the mutant protein has a longer half-life and is stable. The mutant P53 protein that accumulates due to the P53 mutation can be detected in the nucleus of cancer cells (20-22).

There is currently no standard for determining the scoring system for the degree of expression of P53. The scoring system used in this study is based on various existing references (20,21). This study obtained 13 samples (32.5%) from patients with invasive breast cancer who did not show expression of the P53 protein by immunohistochemical examination. This finding was similar to those of studies conducted by Gonzalez-Angulo et al. (20) and Lacroix et al. (21), both of which reported that P53 expression was detected in less than 75% of breast cancer patients. The reason is that not all mutations produce stable proteins (i.e., long half-life before being degraded by protease enzymes), and some mutations produce truncated proteins (i.e., unstable and easily degraded proteins), meaning that they cannot be detected by immunohistochemical examination.

BC samples in this study that did not express P53 may have had a type of mutation that produced an unstable P53 protein. As a result, the protein may have easily degraded and, therefore, not been detected by immunohistochemical examination.

Based on *table 3*, our study found the distribution of BC patients by histopathologic grading group to be highest in the “high” grading (27 samples; 67.5%). The remainder was “moderate” grade (12 samples; 30%) and “low” grade (1 sample; 2.5%). At the same time, we found the highest P53 expression group data in the positive expression level of 33 samples (82.5%), and the remaining negative expression levels were found in 7 samples (7 samples; 5%). The statistical test results with Spearman's rho test obtained a high significance value ($p \leq 0.001$) for the relationship between the degree of expression of P53 and the histopathological grade of BC, with the correlation coefficient r = sense positive. This shows that high grades of BC are more common in patients with positive P53 expression (82.5%), while low and moderate grades of BC are more common in patients with negative expression of P53.

These results also indicate that P53 mutations are still low in number at a low degree of malignancy, so the accumulated mutant P53 levels are also low. This shows that the higher the grading, or the more virulent the mutations, the more P53 mutations there will be, resulting in a more significant accumulation of mutant P53. Previous studies have extensively investigated the relationship between P53 expression and BC malignancy grade, both with IHC and PCR techniques. Another study found a relationship between the expression of P53 and the histopathological grade (20,21,23).

The P53 protein works to maintain the stability and integrity of the genome; its mechanism of action is to prevent cells with damaged DNA from continuing to proliferate and to activate apoptosis if the damage cannot be repaired (24,25). Previous studies have shown that the positive expression of P53 is associated with high degrees of differentiation, increased mitotic activity, and aggressive behavior (24-26). This is consistent with our study: we found that positive expression of P53 is more common in samples with poor differentiation.

The P53 gene plays a role in inhibiting angiogenesis and metastasis. Serpin, which is part of the Maspin family (breast serine protease inhibitor), plays a role in inhibiting invasion, angiogenesis, and P53 influences metastases, its mechanism of action (27-29). Furthermore, another metastasis suppressor protein is also known, namely KAI1, the mechanism of action of which is also regulated by P53. This clearly shows that if a P53 mutation occurs, the mechanism of action is disrupted, facilitating tumor cell metastasis (20-22,30,31).

Based on Table 4, the highest simultaneous expression of P53 and Ki-67 in both positive expressions was 29 samples (72.5%), and the lowest was a negative expression of P53 and a positive expression of Ki-67 in 3 samples (7.5%). Spearman's rho correlation test results obtained a significance value of p = 0.059 in the test of the relationship between the level of qualification of expression of P53 and the expression of Ki-67 of this study sample. The value is greater than the threshold defining statistical significance, meaning that

there is no significant relationship between the skill level of P53 expression and Ki-67 expression. The value of the correlation coefficient is low, at 0.302 in the negative direction. We can interpret that the strong expression of the P53 gene did not show an increase in the level of expression of Ki-67 in 1 sample simultaneously. This result is consistent with the results of other studies which also examined the relationship between the expression of P53 and Ki-67 and did not obtain significant test results. Lebe et al. (32) conducted a study on the relationship between the expression of P53 and Ki-67 in invasive micropapillary mammary carcinoma and their relationship with other prognostic parameters in 32 cases. They found no significant relationship between P53 expression and Ki-67. In their research, Lebe et al. (32) obtained Ki-67 expression in 62.5% of patients and P53 expression in 75% (24 out of 32 cases).

A limitation of this study is the small sample sizes. Further research should examine the expression of Ki-67 and P53 expression associated with other prognostic and predictive factors.

CONCLUSION

The associations observed demonstrate the relationship between Ki-67 and P53 expression with histopathological grading of BC. It is worth considering the use of Ki-67 and P53 as biomarkers for prognostic and predictive markers of BC.

Conflict of interest

All author declare that they have no conflict of interest.

Ethical Statement

The Health Research Ethics Commission of the Faculty of Medicine, Hasanuddin University approved this study.

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