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The association between Caveolin-1 expressions with clinicopathological characteristic of breast cancer

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ABSTRACT

Introduction: Currently, prognostic determination of breast cancer often posed a challenge to oncologists due to variability in many molecular processes and metabolism. Caveolin-1 is caveolae protein that is closely related to tumour metabolism and had been proved to be associated with the level of malignancy in pre-clinical studies, but there is still no clinical evidence about Cavolin-1. Therefore, this study aimed to evaluate the association between the expressions of Caveolin-1 with clinicopathological characteristics of breast cancer.

Methods: An analytical cross-sectional study was conducted in Sanglah General Hospital and Biochemistry Laboratory by using 78 subjects. The clinicopathological characteristics were recorded from medical records while Caveolin-1 expression was determined by IHC.

Results: Caveolin-1 expression was found to be significantly associated with clinical stadium, subtype, histological grade and LVI in bivariate analysis. Multivariate analysis found significant only in three of them with high expression of Caveolin-1 was associated with early stadium, negative LVI and Luminal A subtype.

Conclusion: Caveolin-1 expression is significantly associated with clinical stadium, LVI and subtype in breast cancer with high expression often delineate early stage, negative LVI and luminal A subtype.

Keywords: Breast Cancer, Caveolin-1, Clinicopathology, Prognostic, Biomarker

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INTRODUCTION

Breast cancer is still one of the health problems worldwide and in Indonesia. Globally, breast cancer is the second most cancer and also the most cancer in women. GLOBOCAN estimates that there were 1.67 million new cases of breast cancer in 2012.[1] Breast cancer is also the 5th leading cause of cancer related deaths worldwide with a mortality rate reaching 522,000 in 2012. However, breast cancer is the leading cause of cancer related death in women in developing countries and the number 2 cause of cancer related death after cervical cancer in developed countries.[2]

The prognosis of breast cancer varies greatly around the world. It depends on the age and stage of the cancer at the time of diagnosis, which can vary from 100% survival for stage 1 cancer and up to only 15% in stage 4 cancer.[3] The prognosis also varied according to the age of diagnosis with the highest survivability observed among those who aged between 40-69 years with 5-years survival reached 90%. However, it is lower among younger age range which is only 84.8% survivability.[4] The high risk of HER2+ and Triple Negative Breast Cancer (TNBC) breast cancers at a younger age range might be the reason for the lower survivability in this age group. But in general, the overall survival of breast cancer patients showed an increase to 86.6% in 2010-2011, significantly increased from 52.5% in 1971-1972.[3]

Another important determinant of prognosis in breast cancer is the histological subtype which consists of luminal A, luminal B, HER2+, and Triple Negative Breast Cancer (TNBC).[5] Luminal-A is the most frequent subtype but also subtype with the highest survival rate. In contrast, TNBC has the lowest prevalence compared to the others but also has the worst prognosis.[6] To support the subtype determination, other factors such as clinical stadium, histological grade, and the response toward adjuvant treatments also assessed to better support the prognostic determination of breast cancer patient. However, recent study revealed that even with all of those factors, prognostic determination still far from accurate due to variability in molecular pattern among cancers as well as variability in tumour metabolism.[7]

The interrelation between tumour metabolism and prognosis first revealed by Lisanti et al.[8] group in 2012. They showed that tumor cells tend to have very different metabolism pattern compared to normal ones, depending highly on glycolysis even in the presence of adequate oxygen level. This phenomenon was first reported by Otto Warburg in 1952 and since then known as Warburg's effect.[9] As a result from this kind of metabolism, cancer cells produced a large quantity of lactate as well as free radicals that further

induce molecular changes within tumour cells some of which could be used as prognostic biomarkers.

Caveolin-1 is one of the proteins affected by metabolic changes in cancers. It is an adhesional protein and often found in caveolae. The study found that caveolin-1 interact with many types of signalling proteins, primarily proteins that play an essential role in tumor suppressor signaling. Caveolin-1 is consistently downregulated in breast cancer and it downregulation had been proved to be closely related with the level of Warburg's effect in TNBC.[10,11] In vitro analysis using TNBC cell lines showed that it downregulation often followed with increased invasive capabilities of cancer cells. However, no clinical evaluation regarding the clinical value of Caveolin-1 is ever conducted yet. Therefore, this study aimed to evaluate the relationship between Caveolin-1 expressions with clinicopathological characteristic of breast cancer patients.

Table 1 **The baseline characteristics of research subjects**

Variable	n=78
Age (Mean ± SD) (Yers)	48.59 ± 10.48
Occupation (n, %)	
Housewife	36 (46,2%)
University Teachers	1 (1,3%)
Teachers	4 (5,1%)
Farmers	2 (2,6%)
State Employee	15 (19,2%)
Private Sector	20 (25,7%)
Education (n, %)	
Elementary	19 (24,4%)
Middle School	12 (15,4%)
High School	25 (32,1%)
Bachelor	20 (25,6%)
Master	2 (2,6%)
Menstrual Status (n, %)	
Premenopause	38 (48,7%)
Postmenopause	40 (51,3%)
Contraception (n, %)	
No Contraception	26 (33,3%)
Hormonal	24 (30,8%)
Non-hormonal	28 (35,9%)
Menarche Age (Mean ± SD) (Years)	13.6 ± 1.17
Parity (Mean ± SD)	2.31 ± 1.32
Tumor Location (n, %)	
Right Mammae	47 (60.3%)
Left Mammae	31 (39.7%)
Tumor Quadrant (n, %)	
Upper Outer	15 (19.2%)
Upper Inner	7 (9%)
Lower Outer	13 (16.7%)
Lower Inner	5 (6.4%)
Central	32 (41%)
Areola	1 (1.3%)
Overlapping Site	2 (2.6%)
Unspecified	3 (3.8%)

METHODS

Research Design and Patients Recruitment

An analytic cross sectional study was conducted from July 2017 to December 2017 in Sanglah General Hospital and Biochemistry Department Laboratory, Faculty of Medicine Udayana University. This study has been approved by Ethical Committee of Faculty of Medicine Udayana University with ethical clearance number 1999/UN 14.2.2.VII.14/LP/2018. According to statistical calculation, a minimum of 78 subjects was required for this study. All newly diagnosed breast cancer patients were included as subjects but defective samples, unreadable staining, or incomplete medical records were excluded.

Caveolin-1 Detection

Caveolin-1 expression was determined using immunohistochemistry (IHC) technique using primary rabbit polyclonal antibody against human Caveolin-1 and mouse secondary anti-rabbit antibody. The staining was conducted in biochemistry department and the samples were evaluated using ImageJ software. To distinguish between high and low expression of Caveolin-1, 30% stained area was selected as cut-off points.

Data Analysis

All of clinicopathological and Caveolin-1 expression data were analysed descriptively to determine raw proportion of each data. Then, bivariate analyses using chi-square or fisher's exact test were used to assess the significancy of the differential proportion among subjects groups. Finally, multivariate analysis was used to evaluate independent relationship between all significant clinicopathological data with Caveolin-1 expression. Risk assessment was also conducted and expressed as Odds Ratio and 95% Confident Interval. P-value less than 0.05 was considered as significant.

RESULT

Subject Baseline Characteristics

Among 78 research subjects involved, the mean age of the subjects in this study was 48.59 ± 10.48 years with 38 (48.7%) were premenopausal women. Most subjects have high school education while

Table 1 *Continued*

Variable	n=78
Stadium (n, %)	
I	2 (2.6%)
II	11 (14.1%)
III	43 (55.1%)
IV	22 (28.2%)
Histopathological Grade (n, %)	
I	7 (9%)
II	26 (33.3%)
III	45 (57.7%)
Subtype (n, %)	
Luminal A	20 (25.6%)
Luminal B	33 (42.3%)
HER2	15 (19.2%)
TNBC	10 (12.8%)
Caveolin-1 (n, %)	
High	26 (33.3%)
Low	52 (66.7%)

Table 2 **Bivariate analysis between Caveolin-1 expressions with several clinicopathological characteristics of breast cancer**

Variable	Caveolin-1 Expression		p
	High	Low	
Stadium			
I	2 (100%)	0 (0%)	<0.001
II	9 (81.8%)	2 (18.2%)	
III	14 (32.6%)	29 (67.4%)	
IV	1 (4.5%)	21 (95.5%)	
Tumor Size			
T1	2 (100%)	0 (0%)	<0.001
T2	10 (90.9%)	1 (9.1%)	
T3	10 (50%)	10 (50%)	
T4	4 (8.9%)	41 (9.1%)	
Lymph Node			
N0	10 (62.5%)	6 (37.5%0	0.002
N1	12 (44.4%)	15 (55.6%)	
N2	4 (12.1%)	29 (87.7%)	
N3	0 (0%)	2 (100%)	
Metastasis			
M0	25 (43.9%)	31 (56.1%)	0.001
M1	1 (4.8%)	20 (95.2%)	
Subtype			
Luminal A	12 (60%)	8 (40%)	0.028
Luminal B	9 (27.3%)	24 (72.7%)	
HER2	2 (14.3%)	12 (85.7%)	
TNBC	3 (33.3%)	6 (66.7%)	

the proportions of elementary and undergraduate schools were found to be almost equal. The proportion of contraceptive use (without contraception, hormonal, and non-hormonal) was also found to be almost equal. Judging from the age of menarche, the mean age of menarche was found to be 13.6 ± 1.17 years with a range between 11-17 years. The mean parity in the subjects of this study was 2.31 ± 1.32 with a range of 0-6 children.

Regarding cancer characteristics, 60.3% of subjects had tumours in the right breast with the central quadrant as the dominant quadrant (41.0%). More than half of the subjects (55.1%) had stage III breast cancer and only 2 subjects (2.6%) were found to have stage I. The dominant histological grade was grade III with Luminal B as the dominant subtype. IHC examination results showed that there were 26 subjects (33.3%) with positive caveolin-1 staining. The basic characteristics of the research subjects are summarised in Table 1.

The Association between Caveolin-1 Expressions with Clinicopathological Characteristics of Breast Cancer

The bivariate analysis showed that caveolin-1 was significantly related several clinicopathological characteristics of breast cancer. It was found to be related with clinical stadium and all of its components such as tumor size, lymph node status and metastasis with negative Caveolin-1 expression often related with larger tumor, more positive lymph node, and the presence of metastasis. It also significantly associated with histological grade with higher proportion of high tumour grade among those who have low expression of Caveolin-1.

Regarding tumor subtype, it also revealed that Caveolin-1 expression was significantly related but per component analysis showed that only HER2 expression that significantly associated with Caveolin-1 expression. For TIL, no significant association was found while positive LVI was significantly associated with low Caveolin-1 expression. The later is consistent with the finding in clinical stadium as LVI often related with metastatic capability of the cancer. All of the results of bivariate analysis are depicted in Table 2.

Multivariate analysis of Caveolin-1 expression with Clinicopathological Characteristics of Breast Cancer

To further assess the findings of the bivariate analysis, logistic regression multivariate analysis was used to analyse the independent association between all significant variables. In this analysis, LVI data, clinical stage, histological grade and subtype were included in the analysis. But before starting the analysis, a re-classification of these

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Table 2 *Continued*

Variable	Caveolin-1 Expression		p
	High	Low	
Estrogen Receptor			
Positive	20 (40%)	30 (60%)	0.095
Negative	6 (21.4%)	22 (78.6%)	
Progesterone Receptor			
Positive	12 (32.4%)	25 (67.6%)	0.873
Negative	14 (34.1%)	27 (65.9%)	
HER2 Receptor			
Negative	15 (48.4%)	16 (51.6%)	0.011
1+	8 (44.4%)	10 (55.6%)	
2+	1 (8.3%)	11 (91.7%)	
3+	2 (11.8%)	15 (88.2%)	
Lymphovascular Invasion			
Positive	6 (18.2%)	27 (81.1%)	0.039
Negative	18 (45%)	22 (55%)	
Tumor Infiltrating Lymphocyte			
Moderate to Strongly Positive	9 (24.3%)	28 (75.7%)	0.109
Negative to Low Positive	17 (41.5%)	28 (58.5%)	

Table 3 The result of multivariate analysis between Caveolin-1 Expression and clinicopathological characteristics of breast cancer

Variable	Adjusted OR	95% CI	p
Subtype	0.214	0.055-0.824	0.019
Clinical Stadium	0.066	0.012-0.375	0.002
LVI	0.250	0.072-0.871	0.029
Histological Grade	1.008	0.91-11.167	0.995

variables was carried out. Referring to the results of bivariate analysis, clinical stages were classified into early stages (stage I) and advanced stages (stages II, III, and IV), histological grades were classified as low grade (grade I) and high grade (Grade II and III), while cancer subtypes were divided into two categories namely Luminal A and Luminal B, HER2 and TNBC which were grouped together. The results of the logistic regression analysis are shown in table 3.

The logistic regression results showed that there were significant relationships between caveolin-1 expression with subtype, clinical stage, and LVI. Meanwhile, the relationship between caveolin-1 and histological grade proved to be insignificant. The analysis also showed that the expression of caveolin-1 has a protective effect and decreases the risk toward more severe breast cancer subtypes (Luminal B, HER2, and TNBC), high grade, and advanced stages.

DISCUSSION

Breast cancer is still one of the urgent health problems both worldwide and in Indonesia.[1,2] It still one of the most common cancers in women and contribute significantly toward cancer-related women mortality. Its urgency is more pronounced in developed countries as the prevalence of cervical cancer has been greatly lowered due to effective vaccination program. However, it is the developing contries that face significant challenge regarding breast cancer as preventive, early diagnostic, and prognostic determinations are much less efficient.[3]

In this study, we found for the first time the potential use of Caveolin-1 as prognostic predictor in breast cancers. Our findings answered the knowledge gap about clinical relevancy of Caveolin-1 expression which confirmed the previous reports from pre-clinical studies.[10,11-13] According to the results, it appeared that the expression of Caveolin-1 significantly associated with subtype, clinical stadium and LVI. The high expression of Caveolin-1 preferably found in tumor with early stage, luminal type and negative LVI. This is concordant with previous studies findings about the tumor suppressor nature of Caveolin-1 in breast cancer albeit it can be pro-tumorigenic in some other types of cancer.[10-13]

Low expression of Caveolin-1 often results from oxidative stress, which leads to degradation of Caveolin-1 through autophagy. Loss of Caveolin-1 expression increases oxidative stress and autophagy in the feedforward mechanism. The absence of Caveolin-1 in breast cancer stroma is associated with a poor prognosis, such as recurrence, lymph node metastasis and resistance to tamoxifen.[12] Also, loss of Caveolin-1 induces transforming growth factor-β (TGFβ) production, oxidative stress, autophagy, oxidative glycolysis, AKT activation and has also been associated with poor prognosis in other tumor types. Increased expression and activation of AKT and TGFβ1 are strongly associated with aggressive cancer development. However, one study showed that high expression of Caveolin-1 was associated with increased expression of α-actin smooth muscle which is a marker of fibroblasts. This kind of phenotype of fibroblasts in stromal cells is associated with a poor prognosis, because it enhances invasion and metastasis of tumor cells.[5]

The expression of caveolin-1 is closely associated with metabolic alteration in cancer, primarily the Warburg Effect. The Warburg effect is known to occur at a higher rate in cancer with a higher level of malignancy. In cases of breast cancer, in vitro studies showed that the Warburg effect was most often found in TNBC subtype cell lines than other subtypes.[9,11] Therefore, the expression of

Caveolin-1 often found to be inversely related with the level of proliferation of cancer cells which is the reason why it often found in low level in TNBC as it is the most malignant type of breast cancer. High level of metabolism also increases free radical production due to high rate of uncoupled electron transport chain.[9,11] This phenomenon resulted in electrons from NADH to be received by oxygen still in high-energy form which ultimately produce ROS both as superoxide and OH radical form. These two ROS also contributed to the downregulation of caveolin-1. However, it is known that cancer cells use ROS to transform fibroblasts into Cancer-Associated Fibroblast so ROS also contributes in forming a pro-tumorigenic microenvironment.[14]

According to the result of this study, the findings were indeed consistent with previous studies that stated the expression of caveolin-1 tended to be lower in the HER2 and TNBC subtypes.[11,14] Those studies also stated that the decreased expression of caveolin-1 was associated with lower survival rates in breast cancer patients with both subtypes. Pre-clinical research also confirmed that decreased caveolin-1 expression in TNBC cell lines increased proliferation, MMP-9 production, BCL-2 expression, and stimulates differentiation of fibroblasts into CAF as well as induced production of IL-10 and TGF-β by these cells.[15,16] Judging from these findings, it can be concluded that caveolin-1 can be a marker of the level of malignancy of those two subtypes, especially TNBC.

The novel finding in this study is that the Luminal B subtype also shows caveolin-1 expression patterns that are similar to HER2 and TNBC. However, the data and literature on this topic are still very limited and there are still no studies analyzing the role of caveolin-1 in Luminal B. The theoretical basis that can be used to interpret these findings is that because the level of proliferation and malignancy is higher in Luminal B compared with Luminal A, the same molecular mechanism that occurred in TNBC could also occur in Luminal B and, thus, Caveolin-1 could also potentially be used as biomarker for this subtype.[17-19] However, further investigation and research into the role of caveolin-1 in Luminal B and its differences with TNBC and HER2 are needed to answer this knowledge gap.

CONCLUSION

Caveolin-1 expression is significantly associated with several clinicopathological characteristics of breast cancer such as clinical stadium, subtype, and LVI with high expression of Caveolin-1 marks lower stadium, negative LVI and Luminal subtype. However, further studies are needed to confirm these findings as well as to validate our finding regarding the similarity of Caveolin-1 expression in Luminal B with HER2 and TNBC.

CONFLICT OF INTEREST

All authors declare to have no conflict of interest regarding this article

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AUTHOR CONTRIBUTION

All authors contributed equally in the writing of this article

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