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 a caveolar 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 Caveolin-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 I 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 1971-1972.⁵

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).⁶ 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.⁷ 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.⁸

The interrelation between tumour metabolism and prognosis first revealed by Lisanti et al.⁹ 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.

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 significance 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 | 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%)                         |
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 stage 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 stage 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

| Variable                  | Caveolin-1 Expression |
|---------------------------|-----------------------|
|                         | High (n, %) | Low (n, %) | p     |
| 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%) |      |
| N1                       | 12 (44.4%)  | 15 (55.6%)| 0.002|
| 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%)   |      |
| Luminal B                | 9 (27.3%)   | 24 (72.7%)| 0.028|
| HER2                     | 2 (14.3%)   | 12 (85.7%)|      |
| TNBC                     | 3 (33.3%)   | 6 (66.7%) |      |
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 countries 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.\(^ {51} \) 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

**REFERENCES**

1. World Health Organization. 2014. World Cancer Report. IARC 2015
2. World Health Organization. International Agency for Research on Cancer. GLOBOCAN 2012: Estimated Cancer Incidence, Mortality and Prevalence Worldwide in 2012. Breast Cancer.
3. Quaresma M, Coleman MP, Rachet B. 40-year trends in an index of survival for all cancers combined and survival adjusted for age and sex for each cancer in England and Wales, 1971-2011: a population-based study. Lancet. 2014;6736(14):61396-9.
4. Cancer Research UK. Breast Cancer Statistics. CRUK. 2015 Accessed: 2 Mei 2015
5. Krigsman O, Ropeman P, Zwart W, et.al. A diagnostic gene profile for molecular subtyping of breast cancer associated with treatment response. Breast Cancer Res Treat. 2011; 1007:10549-11
6. Badve S, Dabbas DJ, Schnitt SJ, et.al. Basal-like and triple-negative breast cancers: a critical review with an emphasis on the implications for pathologists and oncologists. Modern Pathology. 2011;24:157–167
7. Bombonati A, Sgroi DC. The Molecular Pathology of Breast Cancer Progression. J Pathol. 2011; 223(2): 307–317
8. Ko YH, Verhoeven HA, Lee MJ, Corbin DJ, Vogl TJ, Pedersen PL. A translational study “case report” on the small molecule “energy blocker” 3-bromopyruvate (3BP) as a potent anticancer agent: from bench side to bedside. J Bioenerg Biomembr. 2012;44(1):163-70
9. Gonzales CD, Alvarez S, Ropolo A, et al. Autophagy, Warburg, and Warburg Reverse Effects in Human Cancer. BioMed Research International. 2014; 12:1-10
10. Wietkiwicz AK, Menezes DW, Dasgupta A, et al. Stromal MCT4 predicts poor clinical outcome in triple-negative breast cancers. Cell Cycle. 2012;11(6), 1108-1117
11. Sotgia F, Martinez-Outschoorn UE, Pavlides S, et.al. Understanding the Warburg effect and the prognostic value of stromal Caveolin-1 as a marker of a lethal tumor microenvironment. Breast Cancer Research. 2011;13(213):1-13
12. Hehlmann S, Cordes N, et al. Caveolin-1: An essential modulator of cancer cell growth and chemoresistance. American Journal of Cancer Research. 2012;1(4):521-530
13. Liu P, Rudicks M, Anderson RG. Multiple Functions of Caveolin-1. The Journal of Biological Chemistry. 2002;277(44):41295-8
14. Bonuccelli G, Whitaker-Menezes D, Castello-Cros R, Pavlides S, Pestell RG, Fatatis A, et.al. The reverse Warburg effect: glycolysis inhibitors prevent the tumor promoting effects of caveolin-1 deficient cancer associated fibroblasts. Cell Cycle. 2010;15;9(10):1960-71

15. Radisky ES, Radisky DC. Matrix Metalloproteinase-Induced Epithelial-Mesenchymal Transition in Breast Cancer. Journal of Mammary Gland Biology and Neoplasia. 2010;15(2):201-212.

16. Seyfried TN, Huysentruyt LC. On the Origin of Cancer Metastasis. Critical reviews in oncogenesis. 2013;18(1-2):43-73

17. Shah R, Rosso K, Nathanson SD. Pathogenesis, prevention, diagnosis and treatment of breast cancer. World Journal of Clinical Oncology. 2014;5(3):283-298.

18. Viale G. The current state of breast cancer classification. Ann Oncol. 2012.;23 Suppl 10:x207-10.

19. Prabawa I, Bharghah A, Liwang F, Tandio D, Tandio A, Lestari A, Budiana I, Manuaba I. Pretreatment Neutrophil-to-Lymphocyte ratio (NLR) and Platelet-to-Lymphocyte Ratio (PLR) as a Predictive Value of Hematological Markers in Cervical Cancer. Asian Pacific Journal of Cancer Prevention. 2019;20(3):863-868. doi: 10.31557/APJCP.2019.20.3.863