Breast Disease **40** (2021) S115–S117
DOI 10.3233/BD-219017
IOS Press

Lipocalin 2 could predict circulating MMP9 levels in patients with breast cancer

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Abstract.
OBJECTIVES: Breast cancer is the most prevalent carcinoma found in Indonesian women, and its incidence remains high worldwide. Lipocalin 2 has been linked with the progression of breast cancer. Matrix metalloproteinase 9 (MMP9) is an enzyme that has an important role in angiogenesis. We investigated the relationship between lipocalin 2 and MMP9 and the ability of lipocalin 2 for predicting MMP9 levels in female patients with breast cancer.
METHOD: A total of 55 female patients with breast cancer were enrolled in this cross-sectional study. Lipocalin 2 and MMP9 were measured by enzyme-linked immunosorbent assay.
RESULTS: Lipocalin 2 was significantly correlated with MMP9 levels ($r = 0.756$, $p < 0.001$). Lipocalin 2 levels could describe the MMP9 levels ($\beta = 0.76$, $p < 0.001$, $R^2 = 56.9\%$).
CONCLUSION: Higher lipocalin 2 levels in female patients with breast cancer indicate higher MMP9 levels. Lipocalin 2 can be used to predict MMP9 levels.

Keywords: Lipocalin 2, MMP9, breast cancer, female

1. Background

Breast cancer has the highest prevalence among other types of cancer in adult females. In women in the USA, new breast cancer cases are the highest compared to other cancer types, while the estimated death due to breast cancer is ranked second after lung and bronchial cancer [1]. In Indonesia, the incidence of breast cancer was highest (30.9%) compared to that of other types of cancer among the female population in 2018 [2].

Lipocalin 2, also known as neutrophil gelatinase-associated lipocalin (NGAL), is a secreted glycoprotein of the adipokine superfamily produced by various cells and tissues, including neutrophils adipose, bone marrow, and spleen. Neoplastic tissues from several organs, including the breast, colon, lung, express higher lipocalin 2 levels compared to the normal tissues [3–5]. Lipocalin 2 promotes breast cancer progression [6]. Conversely, inhibiting lipocalin 2 activity via inhibitory monoclonal antibodies reduced the metastasis progression of breast cancer in mice with breast tumors [7]. Therefore, lipocalin 2 is proposed as a potential target in the therapy of breast cancer metastasis [8].

Matrix metalloproteinases (MMPs) are a family of endopeptidases containing zinc and can degrade various components of the extracellular matrix. MMPs have important roles in the development of angiogenesis [9]. MMP9 is a member of MMPs with gelatinase activity and the ability to break down type IV collagen of the basement membrane; thus, it has an important role in tumor cell invasion and metastasis. Patients with breast cancer had higher serum MMP9 levels than those

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Table 1
Characteristics of the patients with breast cancer

Variable	*n* (%)	Mean ± SD	Median	Min.	Max.
Stage					
Early	11 (20)				
Late	44 (80)				
Age (years)		43.64 ± 9.05	42	31	68
Lipocalin 2 (ng/mL)		104.86 ± 73.51	79.58	33.43	347.95
MMP9 (ng/mL)		766.34 ± 408.43	671.98	159.25	2400.81

with benign breast disease and normal controls [10]. Higher plasma MMP9 levels have been associated with lymph node metastasis and higher tumor stage [10,11]. Lipocalin 2 may be present with MMP9 in the form of a complex via the disulfide bridge and increases the gelatinolytic activity of MMP9 [1].

In the present study, we investigated the relationship between lipocalin 2 and MMP9 and the ability of lipocalin 2 for predicting MMP9 levels among female patients with breast cancer.

2. Methods

2.1. Study population and ethics approval

This cross-sectional study was conducted from June 2013 to September 2013. The ethical recommendation was approved by the Health Research Ethical Committee of the Faculty of Medicine, Hasanuddin University, Makassar, Indonesia, following the Declaration of Helsinki.

The participants were women with breast cancer hospitalized at Dr. Wahidin Sudirohusodo Hospital, Makassar, Indonesia. Breast cancer was confirmed based on the histopathological diagnosis. Patients who had previous breast cancer therapy, recent infection and a history of kidney and liver disorders were excluded from the study.

2.2. Laboratory procedures

A venous blood sample (3 ccs) was obtained from each participant in a tube without anticoagulant, followed by serum separation with 5-minute centrifugation at 300 pm. The serum was stored at −20 °C until lipocalin 2, and MMP9 testing was performed. Lipocalin 2 and MMP9 were measured by enzyme-linked immunosorbent assay (ELISA) (Quantikine, Minneapolis).

Table 2
Correlation between Age and Lipocalin 2 with MMP9 levels

Age		Lipocalin 2	
r	*p**	*r*	*p**
−0.163	0.234	0.756	<0.001

*Spearman correlation test.

2.3. Statistical analysis

Data distribution normality was tested with the Kolmogorov-Smirnov test. The correlation between parameters was analyzed with either the Pearson or Spearman test. The association between lipocalin 2 and MMP9 levels was determined with linear regression testing.

3. Results

A total of 55 women with breast cancer were recruited to this study, consisted of 11 patients (20%) with early-stage disease (stage 1 and 2) and 44 patients (80%) with late-stage disease (stage 3 and 4). The mean patient age was 43.64 ± 9.05 years (Table 1).

There was a significant correlation between lipocalin 2 with MMP9 levels ($r = 0.756$, $p < 0.001$), but age was not correlated with MMP9 levels (Table 2).

Linear regression analysis showed that lipocalin 2 could describe 56.9% of serum MMP9 levels (Table 3).

4. Discussion

Lipocalin 2 has been used to predict the progression of several cancers, including breast cancer. Lipocalin 2 is overexpressed in breast cancer cells and

Table 3
Linear regression analysis of Lipocalin 2 and MMP9 levels

	Variable	β	p
Model R^2 = 56.9%	Lipocalin 2	0.76	<0.001

can upregulate several mesenchymal markers, including fibronectin and vimentin, and downregulates the epithelial marker E-cadherin, resulting in increased tumor cell motility and invasiveness [6]. The HER2 upregulates the high expression of lipocalin 2 in breast cancer cells–phosphoinositide 3-kinase (PI3K)–AKT–NF-κB pathway [7].

Here, we show that serum lipocalin 2 was significantly correlated with circulating MMP9 levels in women with breast cancer. Interestingly, the highlight of our findings is that lipocalin 2 levels alone could predict 56.9% of serum MMP9 levels. There are several possible explanations for this. Lipocalin 2 may form a complex with MMP9 via a disulfide bridge, rendering MMP9 less prone to degradation by tissue inhibitor of metalloproteinases 1 (TIMP1) due to the protective effect of lipocalin 2 on the structural stability of MMP9 in the form of the complex [1]. Lipocalin 2 can induce increased MMP9 enzymatic activity, thus contributing to MMP9 levels. Lipocalin 2 also regulate pro-MMP9 by inducing the activation of pro-MMP9. These actions may explain the strong effect of serum lipocalin 2 on MMP9 levels in patients with breast cancer [4]. A study on gastric cancer reported that hepatic growth factor produced by mesenchymal cells upregulated lipocalin 2 expressions. The upregulation of lipocalin 2 regulated MMP9 expression through the PI3K–AKT–NF-κB path [12].

This study has some limitations. First, the cross-sectional design cannot describe the causality interaction between the variables observed. Second, the number of patients in this study was limited; therefore, future studies should involve a larger sample size.

5. Conclusion

Higher lipocalin 2 levels in women with breast cancer indicate higher MMP9 levels. Lipocalin 2 can be used for predicting MMP9 levels.

Conflict of interest

None.

Funding source

None.

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