Analysis of Serum Vitamin D and Major Histocompatibility Complex *Class* I-Related Chain a (Mica) Levels in Patients With Breast Carcinoma

Asvin Nurulita[1], Budi Mulyono[2], UmiSolekhah Intansari[2], Mansyur Arif[1]

[1]Clinical Pathology Department, Faculty of Medicine, Hasanuddin University, RSUP Dr. Wahidin Sudirohusodo Makassar, [2]Clinical Pathology Department, Faculty of Medicine Gajah Mada University, RSUP Dr. Sardjito Yogyakarta

Abstract

Introduction: Breast carcinoma is the most deadly cancer in women worldwide, with an incidence rate of 26 cases per 100,000 women in Indonesia. Treatment and prognosis is very dependent on the stage of breast carcinoma. Vitamin D can inhibit proliferation and invasive cancer cells, induction of differentiation, apoptosis and promotion of angiogenesis. Major Histocompatibility Complex class I - related chain A (MICA) plays a role in reducing the expression of NKG2D receptors in breast carcinomas so that they can be targeted for treatment of breast carcinoma treatment. The aim of this study was to determine vitamin D and MICA levels in serum patients with non-metastatic and metastatic breast carcinoma.

Method: We examined 86 samples of breast carcinoma patients (44 non-metastatic samples and 42 metastatic samples) with a range of 29 - 68 years old. Vitamin D levels were examined by the Enzyme Linked Flourescent Assay (ELFA) method while the MICA levels were examined by Enzyme Linked Immunoabsorbant Assay (ELISA).

Result: There were significant differences in mean vitamin D levels in non-metastatic and metastatic breast carcinomas (29.88 ng/mlvs19.06 ng/ml; $p < 0.01$).Serum MICA levels in patients with non-metastatic breast carcinoma were lower (333.52 pg/ml) than in metastatic breast carcinoma

(333.52 pg/mlvs528.71 pg/ml; $p < 0.01$).A negative correlation was found between vitamin D levels and MICA in both groups (Pearson correlation, p: -0.58).

Conclusion: Metastatic breast carcinoma had lower vitamin D levels and higher serum MICA levels than non-metastatic breast carcinoma.

Keywords: *Breast carcinoma, Vitamin D, MICA.*

Introduction

The risk factors for breast carcinoma include genetic factors (gene mutations of BRCA1, BRCA2 and *tumor protein p53*), hormonal, history of hormonal therapy, environmental factors such as radiation exposure, social biology factors (gender, age and race), nutrition factors (a high-fat diet, caffeine and red meat), physiological factors, family factors, alcohol consumption, obesity, a weak immune system and smoking[1,2]. Genetic and various risk factors possessed as carcinogenesis, causing breast epithelial cells to proliferate, polarity and excessive differentiation. Cellular components of the tumor stroma consist of myofibroblasts, vascular cells, *leukocytes infiltrate*, and mesenchymal cells. Tumor-infiltrating Leucocyte (TIL) is also one of the causes of carcinoma[3].

Patients with breast carcinoma are generally diagnosed when they are at an advanced stage leading to an increase mortality and morbidity[4]. The main factors that contribute to the high mortality and morbidity of breast carcinoma are metastasis, where 400,000 patients in the world died each year, the most common metastases are bone metastases (30-85%) with an mean life expectancy of only 25-72 months[5].

Major Histocompatibility Complex class I-related chain A (MICA) is a membrane-bound protein that functions as a ligand to stimulate receptor activation of Natural Killer Group 2 Member D (NKG2D). Major Histocompatibility Complex class I - related chain A includes MIC family members expressed in various types of malignant tumors such as breast, colon, liver and melanoma. MICA expression can be triggered by an active pathway in various pathophysiological conditions such as infection and oncogenic transformation (often expressed in epithelial tumors)[6].

Holdenrieder[7] showed that serum MICA levels increased significantly in malignant patients (breast, lung, gastrointestinal, gynecological, kidney, and prostate carcinoma) compared to patients with benign tumors and healthy controls. Serum MICA levels in carcinoma patients are associated with stage and metastasis[7]. Roshani[8]in Iran showed that serum MICA has an important role in reducing the expression and presentation of NKG2D receptors in breast carcinoma patients and recommends that MICA be targeted for treatment of breast carcinoma.

Calcitrol inhibits the expression of cytokines IL-1, IL-6, IL-12, and TNF-α and inhibits the expression of Major Histocompatibility Complex (MHC) in both class I and class II Seydelfound that administration of Vitamin D2 was able to suppress MICA expression in hepatocellular carcinoma patients[9-11].

Research on serum vitamin D and MICA levels in breast carcinoma in Indonesia, especially in Makassar as long as the researchers' knowledge has not been done so we are interested in conducting research on the analysis of serum vitamin D and MICA levels in metastatic breast carcinoma and non-metastatic breast carcinoma.

Materials and Method

A. **Study Design:** This study aims to find out the differences serum vitamin D and MICA levels in patients with non-metastatic and metastatic breast carcinoma. This study was a descriptive analytic study with a cross sectional approach.

B. **Place and Time of Study**

1. **Study Place**

a. Outpatient Installation and Inpatient Installation of Surgical Oncology RSUP Dr. Wahidin Sudirohusodo Makassar and other networking hospitals for sampling.

b. Installation of Clinical Pathology Laboratory RSUP Dr. Wahidin Sudirohusodo, and FKUH Research Unit/RSPTN Hasanuddin University for examination of serum Vitamin D and MICA levels.

2. **Research Time:** The study was conducted from January-May 2019.

C. **Inclusion and Exclusion Criteria**

1. **Inclusion Criteria:** a. Research subject group: adult women (aged 20-70 years) who were diagnosed with breast carcinoma by the clinician in the Surgical Oncology Department based on history taking, physical examination, laboratory examination, radiological examination and/or histopathology and had never received chemotherapy, radiotherapy and surgery.

b. Willing to participate in research by signing an informed consent.

2. **Exclusion Criteria:**

a. Patients detected suffer from other primary malignancies.

b. Breast carcinoma patients diagnosed with infectious diseases.

c. Lipemic, icteric or hemolytic specimens.

d. Insufficient sample volume.

e. Incomplete data.

D. **Research Permit and Ethical Feasibility:** Every action in this study was conducted with the permission and knowledge of the patients which were used as research samples through an informed consent sheet and stated to fulfill ethical requirements to be carried out from the Health Research Ethics Commission of the Faculty of Medicine, Hasanuddin University-Hasanuddin Hospital-RSUP Dr. Wahidin Sudirohusodo Makassar (KEPK FKUH-UH-RSWS).

E. **Research Process:**

a. Record identity of patients who meet the inclusion criteria and provide a complete explanation of what will be done to them and ifthey agree they will fill out and sign an informed consent.

b. Subjects who fulfilled the inclusion criteria were sampling of 3 ml venous blood. The serum is obtained after a blood-filled tube forms a clot for 30 minutes at room temperature and then centrifuged for 20 minutes at 3000 rpm. Samples were collected enough, stored at 80°C for 12 months, when examined the samples were thawed at 25°C before analysis.

c. Laboratory examination:

1. Vitamin D levels were examined using the Vidas Biomerieux device in principle with the Enzyme Linked Flourescent Assay (ELFA) using the Biomereux reagent VIDAS 25OH Vitamin D.

2. MICA levels were examined using the Microplate Reader Multiscan FC Thermoscientific with the principle of an Enzyme-Linked Immunoabsorbant Assay test (ELISA) using the Human MICA ELISA reagent kit (Bioassay Technology Laboratory).

F. Analysis Method: Data distribution was tested for normality using the Kolmogorov-Smirnov test. If the distribution of data is normal, the serum vitamin D and MICA levels between the two groups (metastatic and nonmetastatic breast carcinoma) were tested using c. The difference was found to be significant if the value of $p<0.05$. Correlation test between serum vitamin D and MICA levels was tested using the Pearson correlation test. Correlation was stated as significant if the value of $p<0.05$.

Results and Discussion

The subject of this research was enrolled for 5 months, breast carcinoma patients who were treated at RSUP Dr. Wahidin Sudirohusodo Makassar and fulfilling the inclusion criteria were 88 patients. As many as 2 patients were excluded, due to incomplete data, so that the data included in the analysis were 86 samples. Patients with non metastatic breast carcinoma were 44 patients and metastatic breast carcinoma were 42 patients. The age of the subjects was 33-76 years, with a median of 50 years. Breast carcinoma patients aged <40 years are around 2.33%, and > 70 years are around 3.49%. This study showed that most breast carcinoma patients in RSUP Dr. Wahidin Sudirohusodo Makassar were in the age group 40- 49 years (Table 1).

Table 1. Characteristics of Study Population

Variable	**Number of Samples {n (%)}**		
	Non Metastatic Breast Carcinoma	**Metastatic Breast Carcinoma**	**Total**
Aged (Year)	44 (51.2)	42 (48.8)	86 (100)
<40	1 (1.16)	1 (1.16)	2 (2.33)
40-49	23 (26.74)	17 (19.77)	40 (46.51)
50-59	11 (12.79)	22 (25.58)	33 (38.37)
60-69	6 (6.98)	2 (2.33)	8 (9.30)
>70	3 (3.49)	0 (0.00)	3 (3.49)
Histopathological Grade			
Adenocarsinoma	1 (1.16)	6 (6.98)	7 (8.14)
IDC	27 (31.40)	11 (12.79)	38 (44.19)
IDC Low Grade	6 (6.98)	6 (6.98)	12 (13.95)
IDC Moderate Grade	1 (1.16)	5 (5.81)	6 (6.98)
IDC High Grade	9 (10.47)	12 (13.95)	21 (24.42)
Papillary Karsinoma	0 (0.00)	2 (2.33)	2 (2.33)

Source: Primary Data

Description: n = Number of samples; IDC = Invasive Ductal Carcinoma.

Hadi[12] found that breast carcinoma patients in developing countries were found in a younger population than in western countries with a majority of productive age (20-50 years). According to the CDC, breast carcinoma at < 45 years can be caused by several risk factors such as a history of breast or ovarian carcinoma, genetic factors and a positive family history.[13]

The serum vitamin D levels obtained were normally distributed by the Kolmogorov-Smirnov test. The mean serum vitamin D level in patients with non metastatic breast carcinoma is 29.88 ng/ml, with a value range of 9.7-33.7 ng/ml. The mean serum vitamin D level in patients with metastatic breast carcinoma is 19.06 ng/ml with a value range of 10.5 - 58.7 ng/ml. Comparison of meanvitamin D levels between the two groups was tested using the t-test. There were significant differences in the mean vitamin D levels in the non metastatic and metastatic breast carcinoma groups with a value of $p < 0.01$ (Table 2).

Table 2. Comparison of serum vitamin D levels in nonmetastatic and metastatic breast carcinoma groups.

Group	Vitamin D Level (ng/ml)			
	n (%)	Mean	SD	P*
Non Metastatic Breast Carcinoma	44 (51.2)	29.88	8.46	<0.01
Metastatic Breast Carcinoma	42 (48.8)	19.06	5.39	

Description: * Independent t-test

The difference in mean serum vitamin D levels in both groups can be seen in Figure 1, where the mean vitamin D level in the nonmetastatic carcinoma group was higher than metastasis.

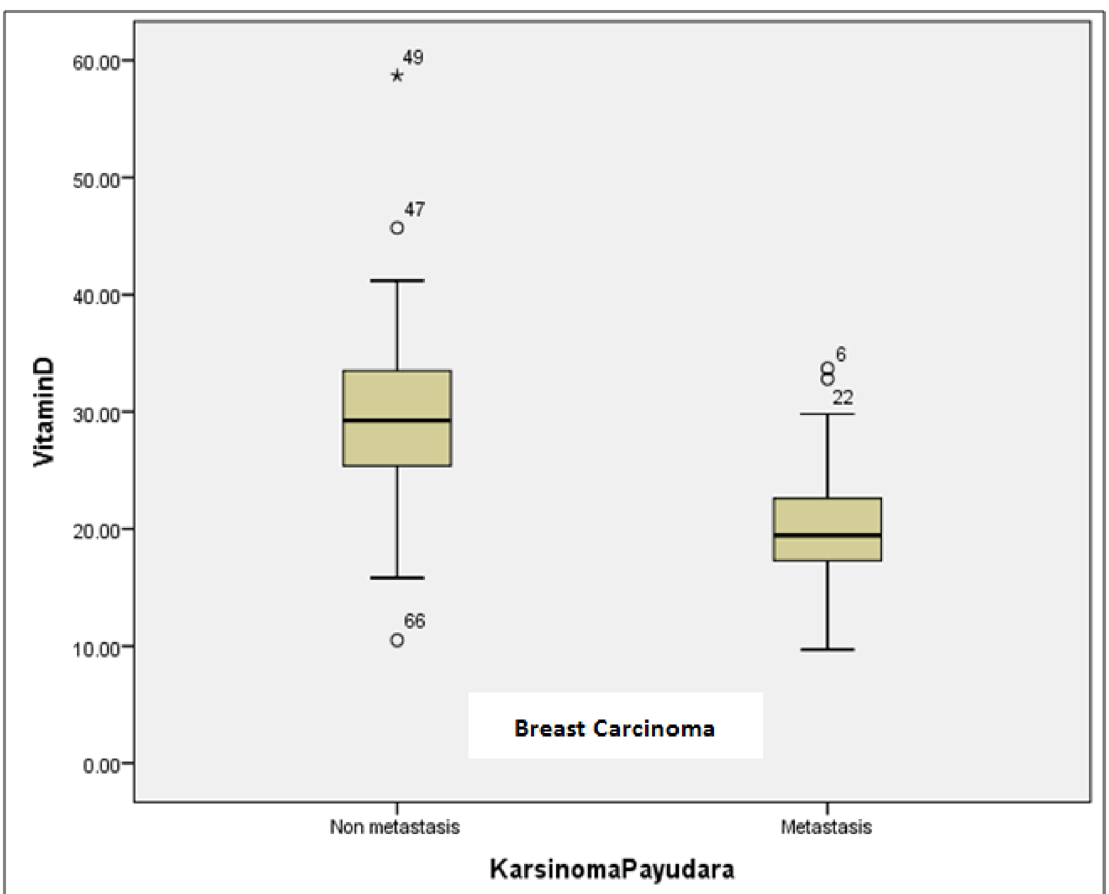

Figure 1. Comparison of mean serum vitamin D levels between nonmetastatic and metastatic breast carcinomas. (source: primary data)

Alco[14] found a median level of 25-OHD 19.76 in patients with breast carcinoma, and a serum 25-OHD level was lower in breast carcinoma patients who died than those who were still alive. Janbabai's study in 2016 found an association between 25-hydroxyvitamin D levels and metastatic breast carcinoma. Patients with distant metastatic breast carcinoma had lower vitamin D levels (12.22 ng/ml) than patients without distant metastasis (23.7 ng/ml)[15,16] showed that there was a relationship between vitamin D deficiency and breast carcinoma, where serum vitamin D levels are significantly lower in breast carcinoma (85.7%) than in control group (55.8%). White[17] found that giving vitamin D can inhibit the progression of breast carcinoma. This further affirm that vitamin D has an antitumor effect that can inhibit proliferation and invasive cancer cells, induction of differentiation and apoptosis, anti-proliferation and antiangiogenesis[18].

The dysregulation of vitamin D metabolism in metastatic breast carcinoma is thought to be due to the effects of tumor paracrine and dysregulation of the 24-hydroxylase enzyme that plays a role in serum vitamin D homeostasis. In addition, microarray analysis shows the relationship of vitamin D with Cyclin-dependent kinase inhibitor P21, which plays a role in controlling the cell cycle. Beckett also found an association of vitamin D levels with overexpression of miR-26 and miR200 associated with increased motility, adhesion and invasion of EMT in metastatic breast carcinoma[19].

Data distribution of serum MICA levels was found to be normally distributed when tested by the Kolmogorov-Smirnov test. The mean serum MICA level in patients with metastatic breast carcinoma is 333.52 pg/ml, with a value range of 177-552 ng/ml. The mean serum MICA level in patients with metastatic breast carcinoma was 528.71 pg/ml with a value range of 364-694 pg/ml. Comparison of mean serum MICA levels between the two groups was tested using the t-test. It was found a significant difference in serum MICA levels in nonmetastatic and metastatic breast carcinoma groups with a value of $p < 0.01$ (Table 3).

Table 3. Comparison of serum MICA levels in nonmetastatic and metastatic breast carcinoma groups.

Group	MICA Level (ng/ml)			
	n (%)	Mean	SD	P*
Non Metastatic Breast Carcinoma	44 (51.2)	333.52	79.40	<0.01
Metastatic Breast Carcinoma	42 (48.8)	528.71	105.13	

Description: * Independent t-test

The difference in mean serum MICA levels in both groups can be seen in Figure 3, where the mean serum MICA level in the metastatic carcinoma group was higher than nonmetastasis.

Major Histocompatibility Complex Class I - related Chain A can be detected in Leukemia patients and tumors in epithelial cells including breast, lung, colon, kidney, ovarian and prostate malignancies[20-21]. Recent study showed that the expression of MICA is limited to the proliferation of epithelial cells but is not expressed in quiescent cells. It has been reported that over-expression of several oncogens can induce MICA expression independently[22]. Increased MICA soluble is detected in patients with pancreatic adenocarcinoma, various gastrointestinal malignancies, *hepatocellular carcinoma*, pulmonary carcinoma, malignant melanoma and various types of leukemia.

The initial stages of tumorgenesis begin with the loss of tumor suppressor gene function (gatekeeper) and activation of oncogenes, resulting in hyperproliferation. At this stage the active E2F transcription factor is needed for the cell cycle to take place, but it can also activate MICA transcription. Some findings indicate a direct relationship between the stages of tumor cell proliferation and MICA induction. Strong proliferative signals can cause DNA damage which results in an upregulation response of MICA and PI3K known to increase proliferation which indirectly also increases MIC transcription[23].

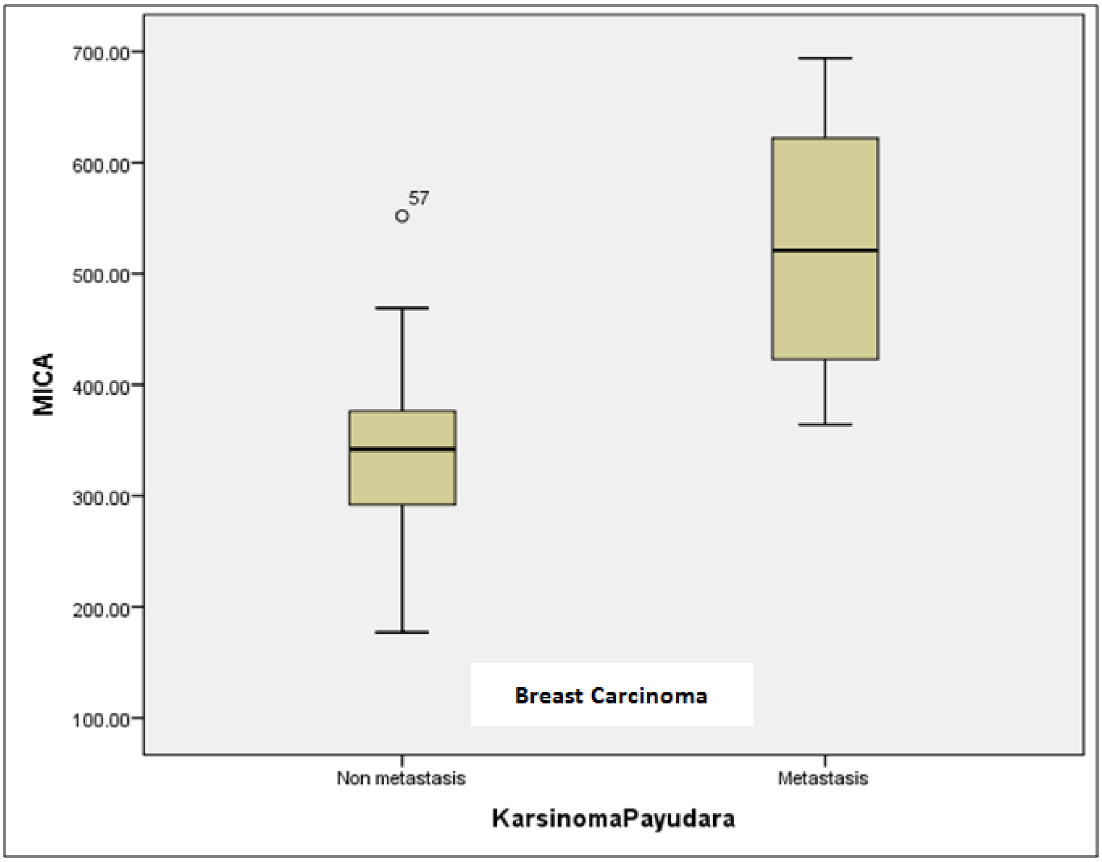

Figure 2. Comparison of mean serum MICA levels between nonmetastatic and metastatic breast carcinomas. (source: primary data)

The study by Cascone[24] in patients with Non Small Cell Lung Carcinoma (NSCLC) found an increase in MICA expression associated with prognosis. MICA overexpression is closely related to DNA damage. The MICA-NKG2D bond can be a NK cell antitumor response costimulator. Tumor cells will protect NK cells through several mechanisms, namely MICA release, proteolytic action of metalloprotease, and suppression of NKG2D regulation on the cell surface.

Correlation of vitamin D and MICA levels in patients with breast carcinoma. followed by the Pearson correlation test. It was found that there was a significant negative correlation between Vitamin D and MICA levels in breast carcinoma patients (p <0.0001) with strong correlation strength (r: -0.58), as seen in table 4. This indicates that the lower the vitamin D level then the higher the MICA level and the risk of metastasis in breast carsinoma will increase.

Table 4. Results of analysis of Pearson correlation of serum vitamin D and MICA levels

Parameter	n (%)	Mean	r*	p*
Vitamin D Level	86 (100)	25.08	-0.58	<0.01
MICA Level	86 (100)	428.85		

Description: * Pearson Correlation Test

Vitamin D can inhibit the expression of Major Histocompatibility Complex (MHC) molecules, both MICA and MICB, costimulatory molecules (CD40, CD80, and CD86), inhibition of dendritic cell maturation and inhibition of proinflammatory cytokines (IL-1 and TNF). In addition, Vitamin D is also able to increase chemotaxisand phagocytosis of monocytes, is cytotoxic to tumor cells and bacteria, and many other effects[10]. The interaction of MICA and NKG2D plays an important role in NK cell activation and tumor immunosurveilens. Chitadze[23] found that NKG2D deficiency was associated with EMT which increased tumor invasion and metastasis.

Conclusion

a. Vitamin D levels in patients with metastatic breast carcinoma are lower than vitamin D levels in non metastatic breast carcinoma.

b. Serum MICA levels in patients with metastatic breast carcinoma are higher than serum MICA levels in non metastatic breast carcinoma.

c. Vitamin D levels were inversely correlated with serum MICA levels in patients with breast carcinoma.

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Source of Funding: Self

Conflict of Interest: Nil

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