

Relationship Between Vitamin D Receptor Expression with Vegf-A in Women with Metastatic Breast Cancer in Dr. Soetomo General Hospital Surabaya

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Abstract

Breast cancer is a malignancy in breast tissue. Breast cancer is the second highest cause of death from cancer in Indonesia. One of the main causes of death in breast cancer is metastatic breast cancer which the 5-year survival rate is only 23.3%. Vitamin D levels and expression of Vitamin D receptors are very important in the process of angiogenesis, especially in endothelial cells of tumor blood vessels. Activation of vitamin D receptors and vitamin D will inhibit the process of neo-angiogenesis thereby reducing the risk of metastasis. Decreased angiogenesis activity can be seen from VEGF-A expression. The research's design is cross-sectional research. The study was done by immunohistochemical staining of breast cancer tissue of patients with metastatic breast cancer for the expression of vitamin D receptors and VEGF-A. In this study a total of 30 patients. It was obtained 20 people (66.7%) aged <50 years and 10 people (33.3%) aged > 50 years. It was also obtained 4 people (13.3%) with liver metastases, 7 people (23.3%) with contralateral breast metastases, 4 people (13.3%) with pulmonary metastases, and 15 people (50%) with bone metastases. The mean expression of vitamin D receptors was 64.71% ± 12.95% and the mean VEGF-A was 66, 96 % ± 7.72 %. It was found that there was a significant relationship between the expression of vitamin D receptors and VEGF-A (p = 0.05). There is a significant relationship between VDR expression and VEGF-A expression in patients with metastatic breast cancer

Keywords: Metastatic Breast Cancer, Vitamin D receptors, VEGF-A, Breast Cancer

1.0 INTRODUCTION

Breast cancer is a malignancy in breast tissue. Breast cancer is the second most common cause of death in all cancers in Indonesia. Breast cancer is also cancer with a large global population of 1 million new cases per year. Breast cancer is 30% of all cancer cases experienced by women. Most breast cancer events occur in women aged over 55 years. (Townsend, 2017). In America, the incidence of breast cancer reaches 2,000,000 in 2018, or 11.6% of all cancer occurrences. Breast Cancer was ranked first inpatients in Indonesia where out of 100,000 women 26 women were treated for breast cancer. In Indonesia, 70% of patients with breast cancer come to the hospital for treatment when they have distant or advanced stage metastases, where patients need more therapeutic modalities. The mortality rate of breast cancer patients in Indonesia is quite high where it reaches 17 deaths in 100,000 populations. (MOH, 2016 WHO 2012).

One of the main causes of death in breast cancer is metastasis so far. According to research data from the American Cancer Society (ACS), the average overall survival rate in breast cancer patients with metastasis since the discovery of distant metastases is only 3 years, it is also mentioned that the 5-year survival rate in breast cancer that has metastasized is 23.3% (Jamnasi et al 2016). Vitamin D has been known to play an important role in calcium homeostasis, bone metabolism, and the functioning of the immune, cardiovascular, and reproductive systems. In some studies, it was found that there is an inverse relationship between Vitamin D and the prevalence of breast cancer. The prevalence of vitamin D deficiency in the world is still quite high reaching 1 billion population. (Manar, 2017). Several laboratory and epidemiological studies have shown that vitamin D levels and vitamin D receptor expression are associated with an increased risk of breast cancer. (Goodwin, 2009). Research conducted by Hamonangan at Dr. Soetomo District Hospital also shows that there are significant differences in blood levels of vitamin D in postmenopausal women with locally advanced breast cancer (LABC) with Metastasis Breast Cancer

(MBC), wherein the group of locally advanced breast cancer (LABC) showed higher mean expression of vitamin D compared to the Metastasis Breast Cancer (MBC) group. (Hamonangan, 2019)

Vitamin D receptors are found in nearly 80% of breast tumor specimens in humans. (WHO, 2016). Research conducted by Chung shows that to inhibit the process of calcitriol angiogenesis requires/depends on vitamin D receptors so that calcitriol cannot work actively without vitamin D receptors. (Chung, 2009). Angiogenesis is a very important factor for tumor growth and tumor cell metastasis. Angiogenesis is a multilevel process that depends on several pro-angiogenic factors including vascular endothelial growth factor (VEGF) and several other growth factors. Calcitriol (1,25 (OH) 2D) can directly inhibit the proliferation of aortic endothelial cells and tumors to stop cell growth and elongation of endothelial cells caused by VEGF. This mechanism is mediated by vitamin D receptors which are found in many cells, one of which is in blood vessel endothelial cells. (James, 2012; Chung, 2009) Among the VEGF subtypes, VEGF-A is a subtype that has the most uses both quantitatively and qualitatively. In one study it was found that VEGF-A plays a very important and angiogenesis role. (Shibuya, 2011).

Vitamin D levels and expression of Vitamin D receptors are very important in the process of angiogenesis, especially in endothelial cells of tumor blood vessels. In the 2009 Chung study, it was found that vitamin D can suppress the proliferation of angiogenesis that appears from VEGF expression. This ability depends on the presence of vitamin D receptors in tumor blood vessels. With the presence of these two components, it is hoped that there will be an emphasis on tumor neoangiogenesis to reduce the incidence of metastases in breast cancer. (Chung et al., 2009) Based on the above thought, this study will analyze whether there is a relationship between the expression of Vitamin D receptors and VEGF-A expression in female patients with metastatic breast cancer (MBC) in Dr. Soetomo

2.0 RESEARCH METHODS

Research in the form of observational analytic research with *cross-sectional* design. Medical record data from breast cancer patients with metastatic breast cancer who met the study inclusion and exclusion criteria. Furthermore, general subject data such as name, age, gender, address, and telephone number are recorded. Other data is recorded according to the data collection form. The results of anatomic pathology in the form of paraffin blocks from breast cancer patients with metastatic breast cancer then underwent an examination of Vitamin D and VEGF-A receptor expression at the anatomical pathology laboratory Dr. Soetomo. To then see whether there is a relationship between Vitamin D Receptor Expression and VEGF-A. Peng processed the data was performed using SPSS 22.0. Data is presented in the form of frequency distribution tables and cross-tabulations. Data from independent and dependent variables in the form of numerical data will be tested using the Pearson correlation test.

3.0 RESULTS

The research subjects consisted of 30 women, with ages most experienced metastatic breast cancer (MBC) is aged under 50 years is 20 patients (66,7%) with a mean of 47.5 ± 9.5 years. In this study, it was found that most breast cancers were found in Grade III as many as 17 patients (56.7%). In this study, it was found that most types of anatomical pathology of the patient metastatic breast cancer (MBC) are a type of Ductal carcinoma in as many as 12 patients (73.3%). The characteristics of the subjects in this study can be seen in Table 1

Table 1 Research Subjects Characteristics

Subject Characteristics	Frequency	Percentage (%)	
Gender	Female	30	100
Age	<50 years	20	66.7
	> 50 years	10	33.3
Grade	I	6	20.0
	II	7	23.3
	III	17	56.7
Anatomical Pathology	Ductal Carcinoma	22	73.3
	Non-Ductal Carcinoma	8	26.7

In this study, in terms of process metastasis then found that patients metastatic breast cancer was observed at t repeated as many as 15 patients (50%). Characteristics of metastasis metastatic breast cancer are shown in Table 2

Table 2 Characteristics of metastasis in metastatic breast cancer

Characteristics of Metastases		Frequency	Percentage (%)
Metastases Location	Liver	4	13.3
	Contralateral Breast	7	23.3
	Lungs	4	13.3
	Bone	15	50.0

After the normality test was done using Shapiro Wilk, the P-value was 0.128, this value indicated that the VEGF-A data was normally distributed.

Table 3 - Average VDR Expressions in Metastatic Breast Cancer

Category	Average	Standard Intersection
VDR	64.71%	12.95%

After the normality test using Shapiro Wilk got a P value of 0.669, this value indicates that the VDR data has a normal distribution.

Table 4 - Mean of VEGF-A expression in Metastatic Breast Cancer

Category	Average	Standard Intersection
VEGF-A	66.96%	7.72%

To test the Pearson correlation between VDR expression and VEGF expression, the correlation coefficient value - 0.352, the negative value f means that the lower the VDR expression, the higher the VEGF expression -A and the correlation is statistically significant with a P-value of 0.05. This result showed that there was a statistically significant correlation between VDR expression with VEGF -A expression in patients with metastatic breast cancer.

Table 5 - Pearson Correlation Test between VDR and VEGF expressions

		Average VEGF-A
VDR Mean	Correlation coefficient	- 0.352
	P value	0.05
Number of subjects		30

4.0 DISCUSSION

Decreased expression of VDR and increased VEGF is associated with high rates of distant metastases in breast cancer. In this study, it was found that there is a relationship between VDR and VEGF-A expression with the incidence of breast cancer metastases. This is consistent with a study conducted by Santos in 2017 which stated that the bond between vitamin D and its receptors (VDR) has been shown to reduce the process of carcinogenesis and metastasis in various pathways. It has been found that the binding of vitamin D with VDR decreases the survival of cancer cells and reduces depression of transporters (GLUT-1) and glycolytic enzymes: GLUT-1, hexokinase II (HKII), and lactate dehydrogenase A (LDHA) (Santos et al 2017). Previous research has shown that increased glycolysis is essential for cancerous growth and metastasis. Thus, inhibition of this important pathway can prevent the

development of cancer. This inhibition of glycolytic enzymes has been shown to reduce cell proliferation and replace apoptosis in cancer cells (Santos et al. 2017). Also, Vitamin-D / VDR binding also has anti-proliferation effects and vitamin D has also been shown to have anti-oxidative anti-invasion, and anti-angiogenesis activity, and most recently also anti-metastasis by targeting inhibition of tumor development differentiation 1 gene (ID1).

The lack of expression of Vitamin D receptors in cells affects TDEC in regulating the growth and formation of new blood vessels or angiogenesis in tumors. Also, the production of COX-2 and HIF-1 which is a factor of proangiogenesis is not well inhibited, causing the process of angiogenesis to continue to occur. This effect will lead to tumor progression, especially in metastasis. (Chung, 2009). In this study, it was also found that there was a correlation between VDR expression and an increase in VEGF-A with the incidence of breast cancer metastases, where the lower VDR expression and increased VEGF-A expression were associated with an increase in the statistically significant increase in breast cancer metastasis.

According to the results of research by Chung in 2009, VDR played a major role in mediating the antiproliferative effects of calcitriol on TDEC and the development of tumor angiogenesis in vivo. TDEC which was isolated from the tumor and implanted in VDR showed very high VDR expression and its growth was inhibited by calcitriol. Calcitriol via VDR induces the termination of the G0 / G1 cell cycle in TDEC. (Chung, 2009). The absence of VDR in TDEC in tumor blood vessels causes aberrant blood vessel growth and tends to be higher in blood vessel growth. This is associated with fewer pericytes and the tumor will contain more angiogenic factors such as VEGF. (Chung, 2009). So with an increase in VEGF, the angiogenesis of the tumor tissue will increase and this will support the process of metastasis.

Angiogenesis is very important for the expansion of tumor growth and tumor cell metastases. This is a multicellular, multicellular process that relies on a variety of pro-angiogenic factors including vascular endothelial growth factor (VEGF), basic fibroblast growth factor (bFGF), and the growth factor of BB homodimer derivatives (BB PDGF). Calcitriol (1,25 (OH) 2D) can inhibit the development of tumor blood vessels necessary for the development of solid tumors and this can occur due to effects on endothelial or epithelial cells. Calcitriol (1,25 (OH) 2D) can directly inhibit the proliferation of aortic endothelial cells and tumors and can stop cell growth and elongation of endothelial cells caused by VEGF.

Stimulation of angiogenesis in response to hypoxia is mediated by hypoxia-inducible 1 factor (HIF-1), which directly increases the expression of several proangiogenic factors including VEGF. Early studies indicate that calcitriol is a potent inhibitor of tumor cells induced by angiogenesis in experimental models. Calcitriol inhibits the formation of induced endothelial cell tubes. Calcitriol and its analogs also directly inhibit endothelial cell proliferation leading to inhibition of angiogenesis. VDR suppresses the expression of IL-8 proangiogenic factors in a way that is dependent on NFkB. The proangiogenic effect of PGE2 produced by COX-2 may be a result of its action to increase the synthesis of HIF-1 α protein in cancer cells.

Therefore, suppression of COX-2 expression by calcitriol provides an important indirect mechanism where calcitriol inhibits angiogenesis, in addition to the direct suppressing effect on proangiogenic factors such as HIF-1 and VEGF. (Aruna, 2010)

5.0 CONCLUSION

There is a significant relationship between VDR expression and VEGF-A expression in patients with metastatic breast cancer.

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