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Comparison of Vitamin D Receptor Expression On Breast Cancer Tissue with Bone Metastasis and Without Metastasis

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Abstract

VDR is a hormone receptor that mediates the regulatory function of vitamin D in proliferation and differentiation. This VDR activity can affect the regulation of several types of vitamin D functions and activities related to cancer in humans. Also, VDR expression can describe the response of tumor cells to 1,25-dihydroxy vitamin D₃ (calcitriol). Based on this thinking, several studies conducted to identify VDR in tumor tissue by immunohistochemical examination are a good method and become a modality for the development of targeted therapies, especially breast cancer metastases. The purpose of this study to determine whether there are differences in the expression of vitamin D receptors in breast cancer tissue with bone metastasis and non-metastatic bone. This research was observational analytic with cross sectional design. The study samples were paraffin blocks of female patients who had been diagnosed with Stage III and IV breast cancer in Dr Soetomo Regional Hospital Surabaya from July to September 2019 who met the study inclusion and exclusion criteria. The independent variables of this study were vitamin D receptors and breast cancer patients who experienced bone metastasis and did not experience metastasis (radiologically proven) as the dependent variable. It was found that there were significant differences in mean VDR expression between groups of breast cancer patients who experienced bone metastasis and those who were non-metastatic ($p = 0.001$). This shows that breast cancer patients with low VDR expression will experience higher bone metastases and vice versa. There are differences in the expression of VDR in breast cancer tissue with bone metastasis and non-metastatic bone. This study found a statistically significant relationship between VDR expression and the incidence of bone metastases in breast cancer.

Keywords: Breast cancer, Vitamin D, Bone metastasis

1.0 BACKGROUND

Breast cancer is cancer with the highest percentage of new cases (43.3%) and the highest percentage cause of deaths (12.9%) in women in the world (Globocan, 2012). Based on the 2013 Basic Health Research data, the prevalence of breast cancer in Indonesia reaches 0,5 per 1000 women (Health Ministry, 2014). Data from the Indonesian Cancer Registry of the "Dharmais" Cancer Hospital, Jakarta, shows an increase in the number of breast cancer sufferers in Indonesia from 1995-2004. There was a change in the trend of cancer types in Indonesia from cervical cancer, which initially was ranked first from 1995 to 2000, and then the second in the 2001-2004 period. From 2001 to 2004, breast cancer ranked first (Sutoto, 2016).

Bone metastasis is a form of metastasis from breast cancer that is often hidden. The quality of life of patients with metastasis is also far worse than that of local carcinomas. Nearly 70% of breast cancer sufferers will experience bone metastasis. Therefore, new strategies continued to be developed in the early detection of bone metastases and their management (Lelekasis et al, 1999).

Several laboratory and epidemiological studies have shown that vitamin D levels and vitamin D receptor (VDR) expression are associated with an increased risk of breast cancer (Goodwin et al, 2009). VDR is a hormone receptor that mediates the function of vitamin D regulation in proliferation and differentiation. This VDR activity can

affect the regulation of several types of vitamin D functions and activities related to cancer in humans. Also, VDR expression can describe the response of tumor cells to 1,25-dihydroxy vitamin D3 (calcitriol). Based on this thinking, several studies conducted to identify VDR in tumor tissue by immunohistochemical examination are a good method and become a modality for the development of target therapy, especially breast cancer metastases.

2.0 RESEARCH METHODOLOGY

The research design used was analytic descriptive research with a cross-sectional research design. The research location is the Ruang Pusat Pengembangan dan Layanan Kanker Dr. Soetomo Regional Hospital, Surabaya and Anatomy Pathology Laboratory Dr. Soetomo, Regional Hospital Surabaya. The study population was paraffin blocks of female patients who had been diagnosed with Stage III and IV breast cancer in Dr Soetomo Regional Hospital Surabaya from July to September 2019. Inclusion criteria included female patients with Stage IV breast cancer based on AJCC staging, aged over 18 years and less than 65 years, paraffin blocks of the patients are available at the PA Laboratory Dr. Soetomo Regional Hospital, patients with metastases and had radiological evidence, had histopathological results of the type invasive ductal carcinoma (IDC) with grade II or III and agreed to follow the research by signing an informed consent. Exclusion criteria included patients with kidney failure, chronic liver disease, general weakness; illustrated in the performance status with a Karnofsky scale measuring $\leq 70\%$, and the patient's medical record is incomplete. The research subjects will be divided into two groups, the research group (bone metastasis) and the control group (without bone metastasis). Both groups will be examined for vitamin D receptors, and then the results in the two groups will be compared.

3.0 RESULT

Table 1. Characteristics of Demography Breast Cancer stadium IV

Category	Metastasis (n=16)	Non Metastasis (n=17)	P
Sex			
Male	0 (0%)	0 (0%)	
Female	16 (48,5%)	17 (51,5%)	
Age (years)			.868
Mean	53,56	56,24	
Minimum	34	40	
Maximum	79	87	
Grading			.009
II	5 (31,3%)	13 (76,5%)	
III	11 (68,8%)	4 (23,5%)	
Luminal Type			.399
Luminal A	2 (12,5%)	0 (0%)	
Luminal B	6 (37,5%)	9 (52,9%)	
Her2-Neu Type	0 (0%)	6 (35,3%)	
Triple Negative	8 (50,0%)	2 (11,8%)	
Ki67			.523
Mean	50,63	41,47	
Low($\leq 14\%$)	1 (6,3%)	2 (11,8%)	
High($> 14\%$)	15 (93,8%)	15 (88,2%)	

Based on Table 1 showed that patients with Stage IV breast cancer in Dr. Soetomo Regional Hospital Surabaya from July to September 2019. In total, there were 33 research subjects, which were divided into 2 groups: 16 subjects who had bone metastasis and 17 subjects who had no bone metastasis.

Table 2. Age group of Breast Cancer Patient

Age Group	N
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31 – 40	2
41 – 50	11
51 – 60	13
61 – 70	3
> 70	4

Based on Table 2. Showed that most patients present at the age of the 4th decade (ages 51-60 years). In the group that experienced bone metastasis, the mean age of the patients was 53.56 years while in the non-metastasis group was 56.24 years. Based on the histopathological grading, breast cancer patients who experienced bone metastasis mostly occupied grade 3 (68.8%) while the rest were grade 2 (31.3%).

Table 3. Correlation of Average of VDR to Histopathologic Grading Metastasis Breast Cancer stadium IV

	N	Average	p
Grade 2	18	77,02 ± 9,55	0,000
Grade 3	15	54,24 ± 10,31	

In contrast to non-metastatic bone patients, most patients showed grade 2 pathology results (76.5%), and the remaining grade 3 (23.5%). Judging from the results of the chi-square test, there is a relationship between higher histopathological grading and bone metastasis in patients with stage IV breast cancer in this study, with a significance value of 0.009. Immunohistochemically examination was performed on each histopathological tissue. In this study, patients with bone metastases found triple-negative breast cancer / TNBC (50.0%) as the most molecular subtypes, followed by Luminal B (37.5%) and Luminal A (12.5%). Whereas patients with no metastasis had the highest type of Luminal B (52.9%), followed by Her2-type (35.3%) and TNBC (11.8%). However, no association was found between molecular subtypes and bone metastases in Stage IV breast cancer in this study. The ki-67 expression has a higher mean value in breast cancer patients with bone metastasis as much as 50.63 while in non-metastatic 41.47. Based on the classification of the expression level, the majority (15 patients) in the bone metastasis group showed a high Ki-67 expression of 93.8%, as well as in the non-metastasis group of 88.2% (15 patients). Judging from the statistical tests, the significance value ($p = 0.523$) showed no relationship between Ki-67 expression and bone metastasis in Stage IV breast cancer in this study.

It was found that the mean VDR expression in patients with grade 2 from histopathology was 77.02 ± 9.55 while patients with grade 3 were 54.24 ± 10.31 . These results illustrate that the higher histopathological grading, the lower the expression of vitamin-D receptors. From the Shapiro-Wilk test results, it was found that the mean VDR expression had a normal distribution ($p > 0.005$).

Table 4. Correlation of Average of VDR to Bone Metastasis Breast Cancer stadium IV

	N	Average	p
Bone Metastasis	16	58.52 ± 12,49	0.001
Non Bone Metastasis	17	74,34 ± 13,42	

Correlation analysis was performed between the mean expression of vitamin-D receptors with histopathological grading. As shown in table 3, there is a significant difference between the expression of vitamin-D receptors and histopathological grading of breast cancer patients with stage IV ($p = .000$). That is, the expression of vitamin D receptors which is lower is associated with increased histopathological grading in breast cancer patients, so the risk of bone metastasis will also increase. This is in line with the results in the previous table (Table 1) where the higher histopathological grading also shows more bone metastasis in breast cancer patients.

4.0 DISCUSSION

In stage IV, breast cancer can do metastases to other organs, one of which is on the bone. In many cases, the frequency of bone metastases is higher than in other places. Nearly 70% of women with breast cancer are found to have bone metastases, and only about 33% have lung or liver metastases. Although it can survive, breast cancer patients with bone metastases will feel pain in a relatively long time, of course, this will reduce the quality of life of patients. This loss of quality can be caused by pathological fractures, spinal cord compression, and metabolic

complications. Bone metastasis makes the economic cost burden significantly increased (2/3 of the cost of treating breast cancer is caused by bone metastasis and is estimated at 3 billion / year).

Bone metastasis in breast cancer is often not identified until the patient feels symptoms of bone metastasis. This can occur because diagnostic tools to date have only limited ability to detect bone stages in the early stages. This phenomenon shows the need for more adequate diagnostic facilities to be able to correctly identify patients who are at high risk of bone metastasis and until now we are still looking for objective markers with high probability that can be used to identify breast cancer patients with bone metastases to improve early diagnostics and appropriate therapy. (Lee, 1998, Lelekasis et al, 1999).

Vitamin D receptor (VDR) is a dependent ligand transcription factor that will bind to calcitriol. VDR is expressed in epithelial, stromal, and immune cells of the breast gland. VDR is involved in cell growth and differentiation in breast cancer tissue. Effects of VDR with stimulation of calcitriol on breast cancer cells including cell cycle arrest, induction of differentiation, and activation of apoptosis. In animal experiments, it has been shown that low VDR expression is associated with increased proliferation and apoptosis of breast epithelial cells (Lee, 1998). Also, several previous studies have shown that VDR expression is associated with various prognostic factors for breast cancer, such as small tumors, low histopathological grading, positive ER-PR, low Ki67 expression, and Luminal subtypes. These studies prove that VDR expression in tumor cells is associated with low mortality in breast cancer (Huss et al, 2019).

In this study, it was found that vitamin D receptor expression had a significant relationship with the incidence of bone metastasis in breast cancer, with a statistically significant value of 0.001 ($p < 0.05$). This shows that the expression of vitamin D receptors is significantly related to the spread of breast cancer to the bone where the lower the expression of vitamin D receptors, the metastasis of breast cancer to bone will increase, whereas the higher the expression of vitamin D receptors, the metastasis in breast cancer to the bone will decrease. Based on these findings, vitamin D receptors can be used as a marker of bone metastasis in breast cancer. This is following the studies conducted by Huss that prove that the majority of tumor cells in breast cancer express VDR in cytoplasmic cells by 76-100%. Huss states that VDR expression in breast cancer correlates with prognosis and mortality from breast cancer (Huss, 2019). Ditsch in his 2012 study stated that VDR expression was related to breast cancer prognosis factors and could be used as one of the therapeutic targets in the treatment of breast cancer.

In this study, the expression of vitamin D in breast cancer with bone metastasis has decreased. This is consistent with the studies conducted by Ooi (2010) and Atoum (2017). Ooi et al conducted a study on mice with breast cancer with bone metastases. In this study, it was found that with vitamin D deficiency, the volume of mice trabeculation decreased by 37.5%, and the growth of cancer cells in the metastatic area increased (in this case bone), as evidenced by the increase in osteolytic lesions through radiological imaging. Atoum et al's research also supports this study by stating that decreased VDR expression results in an increase in neoangiogenesis, and the growth of cancer cells. Based on these studies it can be concluded that VDR expression has a relationship with the incidence of bone metastases in breast cancer.

Tumor characteristics are proven as factors that can predict the prognosis of breast cancer, such as tumor size, lymphocytic status, tumor grading, receptor hormone status, Ki67 expression, and tumor molecular subtypes. In this study, data analysis showed a correlation between high histopathological grading and bone metastasis, with a statistical significance value of 0.009 ($p < 0.005$). There was a statistically significant relationship between low VDR expression and tumor histopathological grading, with a significance value of 0,000 ($p < 0.05$). Similar results by Huss et al. (2019), which prove the link between VDR expression and prognostic factors for breast cancer including histopathological grading, confirm this in our study. However, in Huss's study, it was also found that low VDR expression was also statistically related to poor prognosis of patients with small tumor size, negative ER-PR status, and high Ki-67 expression. Also, VDR expression has a significant relationship with the type of tumor histopathology, where a low VDR is more likely to indicate a type of ductal carcinoma. In the Huss study, if we see from its molecular subtype, it was found that low VDR expression was most commonly found in triple-negative tumors (TNBC).

In this study, no association was found between VDR expression and other prognostic factors for breast cancer, except histopathological grading. This might be due to the limited number of study samples. However, to reduce confounding factors in this study, research subjects who participated in the analysis were limited by several inclusion criteria, including stage IV breast cancer, types of ductal carcinoma histopathology and grade 2-3 histopathology. With these criteria, the results of the analysis of this study which showed a significant correlation

between low VDR expression and high tumor histopathological grading can be considered a poor prognostic factor in patients with stage IV breast cancer with an increased risk of bone metastasis.

Recent studies have reported mixed results regarding VDR expression. The study conducted by Ditsch et al (2012) showed an association of VDR expression with tumor size and lymphogen invasion, but was not related to grading, ER-PR or Her2-neu. The researchers said that the difference in results might also be due to the small number of study samples. However, the study prospectively followed and followed up the research subjects to determine patient outcomes. The study results show that in addition to tumor size, VDR expression is also associated with progression-free survival (PFS). Patients with high VDR expression have better progression-free survival (PFS) and overall survival (OS).

The strength of this research is the histopathological examination which is consistently carried out by the same person, namely an anatomic pathologist, as well as complete data documentation. The weakness of this study is the small number of research samples and less long research time, so that it can be the reason for the results of multivariate analysis that is different from other studies. Therefore, caution and accuracy are needed to interpret the results of this study.

5.0 CONCLUSION

There are differences in the expression of VDR in breast cancer tissue with bone metastasis and non-metastatic bone. There is a statistically significant relationship between low VDR expression and the incidence of bone metastases in breast cancer. There is a relationship between VDR expression and prognostic factors in breast cancer, namely histopathological grading.

Reference

Atoum M, Foad A. (2017). *Vitamin D and Breast Cancer: Latest Evidence and Future Steps*. Breast Cancer: Basic and Clinical Research. 2017; 11:1-8

Globocan. (2012). *Estimated Cancer Incidence, Mortality, Prevalence and Disability adjusted life years (DALYs) Worldwide in 2008*. 2012. IARC Cancer Base No. 11.

Goodwin PJ, Ennis M, Pritchard KI, Koo J, Hood N. (2009). *Prognostic Effects of 25-Hydroxyvitamin D Levels In Early Breast Cancer*. J Clin Oncol 27: 3757–3763.

Huss L, et al. *Vitamin D Receptor Expression In Invasive Breast Tumors and Breast Cancer Survival*. Breast Cancer Research. 2019;21:84.

Health Ministry. (2014). *Data And Information Center*. Jakarta selatan.

Ditsch, K. (2012). *The Influence of Logo Design and Branding on Political Campaigns. Theses*. Bloomington: Indiana University Bloomington.

Lee AV, Hilsenbeck SG, Yee D. (1998). *IGF System Components Asprognostic Markers In Breast Cancer*. Breast Cancer Res Treat 1998; 47:295-302

Lelekasis et al. (1999). *A Novel Orthotopic Model Of Breast Cancer Metastasis To Bone*. Klawer Academic Publisher. 17: 163-170,1999.

Ooi LL, et al. (2010). *Vitamin D Deficiency Promotes Human Breast Cancer Growth in a Murine Model of Bone Metastasis*. CancerRes. 2010;70: 1835-44

Sutoto, MD. (2016). *Dharmais National Cancer Center Indonesia. The role in National Cancer Control Program of Indonesia*. (<https://www.scribd.com/document/45501514/Dharmais>) National Cancer Center Indonesia. The role in National Cancer Control Program of Indonesia) diakses 8 November 2016