



# The Relationship of 25-Hydroxyvitamin D Plasma Levels with Breast Cancer Stadium Assessed from Menopause Status

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## **Abstract:**

*Breast cancer is a type of cancer with high incidence and mortality especially in developing countries. Vitamin D regulates the expression a number of genes involved in the development of cancer cells. The aim of this study is to analyze the relationship between 25-hydroxyvitamin D (25 (OH) D) plasma level with breast cancer stage based on menopausal status. This is an observational research method with cross sectional design. Research subjects were 53 newly diagnosed breast cancer patients and had not received chemotherapy. Menopausal status and stage data were obtained from interviews and medical record data. Levels of 25-hydroxyvitamin D plasma were measured (ELISA) method. The results obtained Stage II, III and IV each have an average level of vitamin D of 28,56 ng/ml (95% CI: 23,61 – 33,52 ng/ml), 28,18 ng/ml (95% CI: 24,49 – 31,87 ng/ml) and 27,86 ng/ml (95% CI: 22,68 – 33,04 ng/ml). The average plasma concentration of 25 (OH) D in pre-menopausal patients is 28,54 ng/ml and average plasma 25 (OH) D levels in post-menopausal patients is 27,79 ng/ml. There was no significant relationship between plasma levels of 25 (OH) D and breast cancer stage in both pre-menopausal and post-menopausal patients.*

## **Keywords:**

*25-hydroxyvitamin D; stage of breast cancer; pre-menopause; post menopause*

## **I. Introduction**

Breast cancer is the most common type of cancer in women with a high mortality rate. In 2012, there were 522,000 deaths from breast cancer worldwide. In Asia, as many as 639,824 cases of breast cancer were recorded throughout 2012. Countries with the highest number of cases include China (187,213 cases), India (144,937 cases), Japan (55,710 cases), Indonesia (48,998 cases) and Pakistan (34,038 cases). The highest mortality rates are in India, China, Indonesia, Pakistan and Japan. (Bray, F (2013); Ghoncheh, M (2016)).

The risk factor for breast cancer is multifactorial, one of which is nutrition. Vitamin D is a fat soluble vitamin which has various functions. Low levels of vitamin D are associated with a number of diseases such as diabetes, cardiovascular disease, osteoporosis, multiple sclerosis and cancer. Several case control studies have reported lower levels of vitamin D in breast cancer patients compared to control subjects. (Atoum, M.F (2017); (Elsoud, M.R.A (2016); (Younus A. (2016)).

This is inseparable from the role of vitamin D as an anticancer agent. Research by Jeong et al. in animals, it has been shown that vitamin D can inhibit the growth and development of breast cancer cells. In invitro 1, 25 (OH) 2D (active vitamin D) and its

analogues have antiproliferative activity that can inhibit proliferation and trigger apoptosis cultured breast cancer cells. (Jeong, Y. (2015); Chen, J. (2014); Duffy, MJ. (2016).

Vitamin D regulates the expression of a number of genes involved in cancer cell development. In the process of proliferation, vitamin D increases the expression of p21 and p27 and decreases the expression of CKDs and cyclin. In the process of apoptosis, vitamin D increases the expression of antiapoptotic proteins. In terms of inhibiting metastasis, vitamin D is able to reduce MMP expression and decrease HIF1 $\alpha$ , VEGF, IL-18 expression. 10 Several studies have reported lower levels of vitamin D in women who have gone through menopause compared to women who have not. Given the large role of vitamin D in the development of cancer cells, vitamin D deficiency in women who have undergone menopause is a risk factor for breast cancer. On the other hand, exposure to estrogen in breast tissue causes women who have not menopause in the age range 45-55 years to be more at risk for breast cancer. (Capatina, C. (2014); Gaugris, S. (2005); Beral, V. (2012)).

Breast cancer stage is a clinical assessment to describe the size of the cancer, its spread and its effects on other organs based on 3 criteria, namely tumor size, lymph nodes and metastases. Judging from the mechanism of action, vitamin D has an influence on the 3 components that determine these stages. Therefore, researchers wanted to see the relationship between plasma 25-hydroxyvitamin D (25 (OH) D) levels with breast cancer stage based on menopausal status.

## **II. Research Methods**

Based on the inclusion criteria, the subjects in this study were women who were diagnosed with breast cancer and had not undergone chemotherapy. A total of 53 subjects were collected from October 2017 - February 2018 at the surgical Oncology clinic of H. Adam Malik Hospital, Medan.

Subjects were interviewed to determine the characteristics of diagnosed age, age of menarche, menopausal status and age, history of contraceptive use and history of breast cancer, while breast cancer stage data were obtained from patient medical records.

The subject's blood was drawn as much as 3 cc. The blood is inserted into the EDTA tube and the plasma is separated. The plasma is then stored at -80c until the vitamin D levels are checked.

Plasma vitamin D levels were checked by ELISA method using 25-hydroxyvitamin D (25 (OH) D) Kit (® DBC Canada). The inspection protocol follows the instructions on the kit.

### **2.1 Ethical Clearance**

This research was approved by the Health Research Ethics Committee, Faculty of Medicine, University of North Sumatra with letter no 473 / TGL / KEPK FK USU-RSUP HAM / 2017. Before conducting interviews and taking blood, all research subjects signed the informed consent after being given an explanation of the aims and benefits of the study.

### **2.2 Statistic Analysis**

Normality test using Shapiro-Wilk Bivariate analysis using the Spearman rank correlation test ANOVA test to assess differences in mean levels of vitamin D in the group stage and menopausal status. The limit of significance set is 5%.

### III. Results and Discussion

#### 3.1 Results

Based on the characteristics of research subjects, it is known that 45.3% of study subjects were in the age range of 40-49 years, 58.5% experienced menarch under 13 years. There were 50.9% patients with post-menopause, and 59.3% with menopausal age 45-50 years, there were 49.1% with a history of hormonal contraceptive use and 17.0% with a family history of breast cancer (Table 1.)

**Table 1.** Characteristics of Research Subjects 15, 16

Characteristics	n (%)
<b>Age</b>	
<40 years	6 (11.3)
40-49 years	24 (45.3)
50-59 years	14 (26.4)
60-69 years	7 (13.2)
≥ 70 years	2 (3,8)
<b>Menarch's age</b>	
≤ 13 years	31 (58.5)
> 13 years	22 (41.5)
<b>Menopause Status</b>	
Pre-Menopause	26 (49.1)
Post-Menopause	27 (50.9)
<b>Menopausal Age</b>	
<45 years	4 (14.81)
45-50 years	16 (59,26)
> 50 years	7 (25.93)
<b>History of hormonal contraception</b>	
There is	26 (49.1)
Not	27 (50.9)
<b>Family history</b>	
There is	9 (17.0)
Not	44 (83.0)

**Table 2.** Average Vitamin D Levels based on Cancer Stage and Menopausal Status

Variable	N	Mean	(95% CI)	<i>p</i>
Cancer Stage				
I	0	-		
II	11	28.56	(23.61 - 33.52)	0.980
III	25	28.18	(24.49 - 31.87)	
IV	17	27.86	(22.68 - 33.04)	

Menopause Status			
		28.54	
Pre Menopause	26	(25.84 - 31.24)	- 0.758
Post Menopause	27	(23.56 - 32.01)	

\* Anova test

**Table 3.** Relationship between Vitamin D Levels and Cancer Stage Based on Menopausal Status

Menopause Status	Stadium	Average Vitamin D Levels	<i>p</i>
<b>Pre Menopause</b>	I	-	
	II	25,967	0.185
	III	30,962	
	IV	26,257	
<b>Post Menopause</b>	I	-	
	II	30,850	0.721
	III	26,063	
	IV	28,555	

\* Anova test

Based on the stage of breast cancer, there were no subjects with stage I, 11 subjects with stage II, 25 people with stage III and 17 people with stage IV. Stages II, III and IV each had mean vitamin D levels of 28.56 ng / ml (95% CI: 23.61 - 33.52 ng / ml), 28.18 ng / ml (95% CI: 24.49 - 31.87 ng / ml), 27.86 ng / ml (95% CI: 22.68 - 33.04 ng / ml). There appears to be a decrease in vitamin levels in line with the increase in breast cancer stage, but it is not statistically significant. Based on menopausal status, there were 26 subjects with pre-menopause and 27 subjects with post menopause. There was no significant difference in vitamin D levels between the menopause and postmenopausal groups, namely 28.54 ng / ml (95% CI: 25.84 - 31.24 ng / ml), 27.79 ng / ml (95% CI: 23 , 56 - 32.01 ng / ml) ( $p = 0.758$ ) (Table 2).

Mean vitamin D levels based on breast cancer stage in the pre-menopausal group, II (25.97 ng / ml), III (30.96 ng / ml), IV (26.26 ng / ml) ( $p = 0.185 > 0.05$ ), and in the post-menopausal group II (30.85ng / ml), III (26.06 ng / ml), IV (28.55ng / ml) ( $p = 0.721 > 0.05$ ). (Table 3). There was no difference in vitamin D levels in the two groups in terms of breast cancer stage.

### 3.2 Discussion

According to Arysha (2020) women are one important element in a family or community. Therefore, women's health, especially their reproductive health, is one of the important health problems. Vitamin D is widely known to have an immunogenic role and

play an anti-proliferative role in the body as well as an endocrine hormone. Vitamin D deficiency is known to be associated with an increase in the incidence of malignancies from the breast, prostate, and colon (Garland, C.F. 2009). Suboptimal vitamin D levels are assumed to trigger cell proliferation, angiogenesis, and metastasis.

In this study, there were no research subjects with stage I. There was no statistically significant relationship between 25 (OH) D levels in each group of breast cancer stages. However, it is still seen that the higher the stage of the study subject, there is a decrease in 25 (OH) D levels. This study is in line with previous research by Karthikayan et al. who found no significant association between cancer stage and levels of 25 (OH) D (Karthikayan, A. 2018). The most common presentation of breast cancer is a lump in the breast that does not cause pain. Some people tend to ignore this symptom. As a result, patients with an advanced clinical stage are a common occurrence. Because everyone's health-seeking behavior is different, this is a confounding factor in clinical staging.

Patients with poorly differentiated breast cancer have lower levels of vitamin D (Karthikayan A, 2018) than patients with moderately differentiated and well differentiated tumors. This suggests that true vitamin D metabolites inhibit proliferation and induce apoptosis and cell differentiation.

Physiologically, changes in a woman's menopausal status are known to have an effect on reducing levels of 25-hydroxyvitamin D (25 (OH) D). This occurs due to changes in diet, lifestyle, insulin sensitivity, and decreased physical activity experienced by menopausal women. In addition, postmenopausal women experience changes in vitamin D metabolism, such as a decrease in the synthesis of vitamin D from the skin or changes in the body's consumption of vitamin D which affects vitamin D status and body physiology (Perez-Lopez FR, 2020). There is also a hypothesis that the presence of estrogen increases activity of enzymes that play a role in activating Vitamin D, decreased levels of estrogen during the menopause transition can trigger symptoms of Vitamin D deficiency (Buchanan, J.R. 1986).

Several studies evaluating the relationship between vitamin D and breast cancer based on menopausal status are still inconsistent (Shin, MH (2002); Lin, J (2007); Knight, JA (2007); Rossi, M (2009)). In this study there was no significant relationship between 25 (OH) D levels and menopausal status in the study subjects. Although it appears that 25 (OH) D levels are lower in study subjects with post-menopausal status. This study is in line with Karthikayan et al. who found no significant relationship between menopausal status and levels of 25 (OH) D (Karthikayan A, 2018). In addition, it is in line with research conducted by Anderson et al. who found no significant interaction between Vitamin D, calcium or menopausal status (Anderson, L.N. 2010).

Unlike the case-control research conducted by Kawase et al. who found that vitamin D and calcium intake were inversely associated with the development of breast cancer risk in all subjects (pre- and postmenopausal). However, vitamin D intake was significantly associated only with the pre-menopausal group of women, and calcium intake was significantly associated with risk in postmenopausal women. This association has been modified by differences in tumor receptor status. So they concluded that vitamin D and calcium decreased the risk of breast cancer in Japanese women and that this relationship differed based on menopausal status and receptor status (ER, PR, HER2).

In this study, there was no significant relationship between vitamin D levels and breast cancer stage based on the menopausal status of the study subjects. This is in line with other studies which state that there is no significant relationship between low vitamin D levels and tumor prognosticity (Imtiaz, S. 2014).

However, in contrast to other studies that stated 25 (OH) D levels were significantly higher in patients with early stage cancer compared to those with advanced or metastatic stages (Palmeri, C. 2006). The relationship between vitamin D levels, breast cancer, and prognostic factors such as tumor stage, grade, size, lymphatic node metastasis and status of hormone receptors were contradictory. Low vitamin D levels are associated with advanced stage, tumor size, and grade in patients with post-menopausal status (Janbabai, G. 2016), similarly to women with premenopausal status but triple-negative (Yao, S. 2017). Vitamin D insufficiency and deficiency are found in tumors with advanced stages and metastases. , many positive lymph nodes, a low proportion of ER +, PR +, and high Ki-67 (de Sousa Almeida, 2017).

The inconsistency of results across existing studies may be related to different sample sizes and limited demographic information related to ethnicity and lifestyle. Menopausal status may be closely related to vitamin D status and vitamin D receptor polymorphisms. In addition, there is a modified influence of environmental factors, such as diet, gene variations that are closely related to vitamin D metabolic pathways such as vitamin D-binding protein, an enzyme that plays a role in activation and degradation of vitamin D. (Atoum, M. 2017).

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