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Comparison of VDR Expression and Blood Vitamin D 1.25 (OH)2 Level between Cervical Cancer Patients and Normal Women

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ABSTRACT

Background: Vitamin D and its receptor (VDR) play a crucial role in the development of gynecological cancers. This study aims to evaluate the VDR expression and blood vitamin D 1.25 (OH)2 levels in cervical cancer patients and healthy women.

Methods: This is a cross-sectional study. In vitro quantitative examination (ELISA) was used for the measurement of vitamin D 1.25 (OH)2 and Sandwich-ELISA was applied for quantitative determination in vitro concentration of Human VDR in serum.

Results: The number of research subjects consisting of 20 cervical cancer patients based on clinical and histopathological results and 20 women without cervical cancer based on clinical and pap smear results. The mean vitamin D 1.25 (OH)2 levels in the cervical cancer group of 209.23 ± 71.476 pg/mL were significantly lower than in the group of normal women of 339.79 ± 139.003 pg/mL (*P*=.001). The mean VDR expression in the cervical cancer group of 5.38 ± 5.478 ng/mL was significantly higher than the group of normal women of 1.89 ± 1.657 ng/mL (*P*=.018). The best cut-off value for vitamin D levels is 239.25 pg/mL (sensitivity 70% and specificity 75%). The cut-off value for VDR expression is 2.23 ng/mL (sensitivity 60% and specificity 75%). Low vitamin D levels increase the risk of cervical cancer incidence by 2.7 times greater, and an increase in VDR expression increases the risk of cervical cancer incidence 2 times greater.

Conclusions: The study results indicated a higher expression of VDR and lower levels of vitamin D 1.25 (OH)2 in cervical cancer compared to normal women. Low levels of vitamin D increase the risk of cervical cancer incidence by 2.7 times greater, and higher VDR expression increases the risk of cervical cancer incidence 2 times greater.

INTRODUCTION

The latest data in 2018 mention that cervical cancer is the fourth most common cancer in women worldwide and the second most common cancer in low- and middle-income countries. Therefore, this is a major cause of morbidity and mortality of cervical cancer [1].

Technological advances have increasingly deepened our knowledge regarding molecular oncology. Vitamin D and its receptor (VDR) play a crucial role in the development of gynecological cancers. The paradigm shift in our understanding of vitamin D as an anti-cancer agent has opened a new horizon to explore how transduction of intracellular signals triggers a group of cellular functions [2]. Vitamin D 1.25 (OH)2 is the active hormonal form of vitamin D. In-vitro studies have suggested various mechanistic pathways in which vitamin D inhibits cervical cancer proliferation and decreases the cervical cancer oncogene, HCCR-1, and increases p21 expression, thereby leading to cell cycle arrest at G1. The vitamin D receptor (VDR) belongs to the superfamily of nuclear receptors and is expressed in a significant number of tumor tissues, indicating that the receptor influences cancer etiology. VDR polymorphisms indicate that the receptor influences cancer etiology. Vitamin D 1α,25(OH)2 has been shown to transcriptionally activate and repress target genes by binding to Vitamin D receptor (VDR). VDR belongs to a superfamily of steroid hormone receptors and is reportedly involved in transcriptional regulation of different genes in a ligand-dependent manner. N-terminal VDR variants are tissue-specifically expressed and required to differentially regulate a network of genes by 1α,25(OH)2 D3 [3].

VDR polymorphisms have been demonstrated to change the activity of the vitamin D-VDR complex. Their correlation to different cancer types has been investigated resulting in heterogeneous results. The most frequently polymorphisms associated with tumorigenesis

are Bsm1, Fok1, Taq1, and Apa1. Several studies have shown the important role of vitamin D and its receptors in gynecological cancer. Preclinical and epidemiological evidence mentions the influence of vitamin D on the reduction in the incidence of gynecological cancer. This is widely known that vitamin D supplements can reduce the risk of cancer [3].

Many previous studies have evaluated the role of vitamin D and VDR in predicting cancers, i.e. colorectal, head and neck, prostate, and breast cancers [4]. There are many studies on vitamin D levels and VDR in non-gynecological cases and only a few on gynecological cases, so the authors are interested in looking further into the role of vitamin D and VDR in cervical cancer, starting by looking at changes in VDR expression and vitamin D 1.25 (OH)2 levels in cervical cancer. No previous studies were determining the VDR expression and vitamin D levels in cervical cancer, especially both in Indonesia and in Dr. Soetomo Public Hospital Surabaya, and this is the basis of a comparative study of VDR expression and vitamin D 1.25 (OH)2 levels between cervical cancer and normal women. This study aims to investigate the VDR expression and vitamin D 1.25 (OH)2 in cervical cancer.

METHODS

This study has been approved by the Ethics Committee of the Dr. Soetomo Public Hospital Surabaya, with the Ethical Clearance Certificate No. 1420/KEPK/VIII/2019 and has been through AE/SAE monitoring and evaluation data entry and the research subject marking by CRU (Clinical Research Unit) No. 070/0002/CRU/I/2020 with 41 research subjects. The research design is an observational analytic study in the form of a cross-sectional observational design with a total sample of 40 subjects taken from October until December 2019. The samples consisting of 20 cervical cancer patients based on the clinical and histopathologic results and 20 women without cervical cancer based on clinical and pap smear cytology results who did not significantly show systemic infection by clinical examination or lab results, not receiving any chemoradiotherapy before and without any history of diabetes mellitus, cardiovascular disease, liver or renal diseases. Primary data were collected using direct interview and examination from the subject (age, family history of cervical cancer, smoking, previous hormonal contraception, sexually transmitted diseases (STD), body mass index (BMI), Vit D level, and VDR Expression), and the secondary data were from medical record e.g. stadium and histopathological reports. Serum was taken from venous blood samples for further quantitative in vitro examination for the measurement of vitamin D 1.25 (OH) 2 by ELISA technique. For the quantitative in vitro determination of Human VDR concentrations in serum, the Sandwich-ELISA technique was applied.

Because of its higher physiological concentration, 25 (OH) D (calcidiol) is usually used in research to evaluate vitamin D levels, and there are already reference values for normal levels. In this study, calcitriol was judged to be related to its work effectively in forming complexes with VDR and retinoid X (RXR) receptors to regulate gene expression in its role related to cervical cancer, where the researchers have not found a cut-off value for normal women from any literature. The best cut-off values for vitamin D level and VDR Expression were picked based on ROC Curve between vitamin D level and VDR Expression by cervical cancer incidence. The best cut-off point for vitamin D level we picked is 239.25 ng/l (sensitivity 70% dan specificity 75%) and the cut-off for VDR expression is 2.23 ng/l. (sensitivity 60% dan specificity 75%).

The statistical analysis from subject characteristics is presented descriptively before applying unpaired T-test for the numerical variable or chi-square for the nominal variable to see mean differences and the proportional difference between normal and cervical cancer groups. We performed the Mann Whitney test to compare vitamin D level and VDR Expression between normal and cervical cancer groups. After knowing the association between vitamin D level, VDR expression, and cervical cancer, multivariate analysis was performed; first, we divide vitamin D level and VDR expression into categorical variables based on the cut-off point chosen. Then, logistic regression was applied to the characteristic variable (P<.05), vitamin D level, and VDR expression to the cervical cancer variable. Odds ratio with P<.05 means significant. All the statistical analyses were performed using SPSS v. 21.0.

RESULTS

The number of research subjects comprised of 41 samples obtained by consecutive random sampling from August 23 to November 18, 2019; one subject was excluded due to a breast cancer history. Of the 40 research subjects, 20 patients had cervical cancer based on clinical and histopathologic results and the other 20 were individuals without cervical cancer based on clinical and pap smear results.

Table 1 shows the mean age of the cervical cancer group is 47 years, significantly older than the mean age of the normal group. In the cervical cancer group, 15% of the patients were in the early stage and 85% in the advanced stage. Meanwhile, in our study, all subjects did not have a family history of cervical cancer, smoking, or consuming vitamin D supplements previously and did not have a history of sexually transmitted diseases. The use of hormonal birth control in the two groups of subjects did not differ significantly. We can conclude that the characteristic that might still complicate this study is the age variable; then, this variable was included in the multivariate analysis.

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Table 1. Research subject characteristics

		Healthy Women		Cervical Cancer		*P*
		Mean±SD	N (%)	Mean±SD	N (%)	
Age		37±7.0		47±11.0		0.001[a]
	Premenopause		18 (90.0)		8 (40.0)	0.001[b]
	Postmenopause		2 (10.0)		12 (60.0)	
BMI		25.0±4.85		24.7±4.39		0.84[a]
	Malnourished		3 (15.0)		2 (10.0)	
	Normoweight		5 (25.0)		7 (35.0)	
	Overweight		1 (5.0)		1 (5.0)	
	Obesity		11 (55.0)		10 (50.0)	
Family History					0 (0.0)	-
	Yes		0 (0.0)		20 (100.0)	
	No		20 (100.0)			
Cigarette					0 (0.0)	-
	Yes		0 (0.0)		20 (100.0)	
	No		20 (100.0)			
STD					0 (0.0)	-
	Yes		0 (0.0)		20 (100.0)	
	No		20 (100.0)			
Hormonal contraception						0.76[b]
	Yes		6 (30.0)		7 (35.0)	
	No		14 (70.0)		13 (65.0)	
Vit D supplement						-
	Yes		0 (0,0)		0 (0.0)	
	No		20 (100.0)		20 (100.0)	
Stage						
	Early-stage		0 (0.0)		3 (15.0)	
	Advance stage		0 (0.0)		17 (85.0)	

BMI: body mass index, STD: sexually transmitted diseases
[a]Independent T-Test
[b]Chi Square

Table 2. Differences in VDR expression and vitamin D 1.25 (OH)2 levels between two groups of subjects

Group	N	Mean±SD Vit D	*P*	Mean±SD VDR	*P*
Normal	20	339.79±139.003	0.001[a]	1.89±1.657	0.018[a]
Cervical Cancer	20	209.23±71.476		5.38±5.478	

[a]Mann-Whitney Test

From **Table 2**, it is apparent that the mean vitamin D 1.25 (OH)2 levels in the cervical cancer group were significantly lower compared to the group of normal women, respectively 209.23 ± 71.476 pg/mL and 339.79 ± 139.003 pg/mL, and the VDR expression in the cervical cancer group was significantly higher than the group of normal women (*P*= .018), respectively 5.38 ± 5.478 ng/mL and 1.89 ± 1.657 ng/mL.

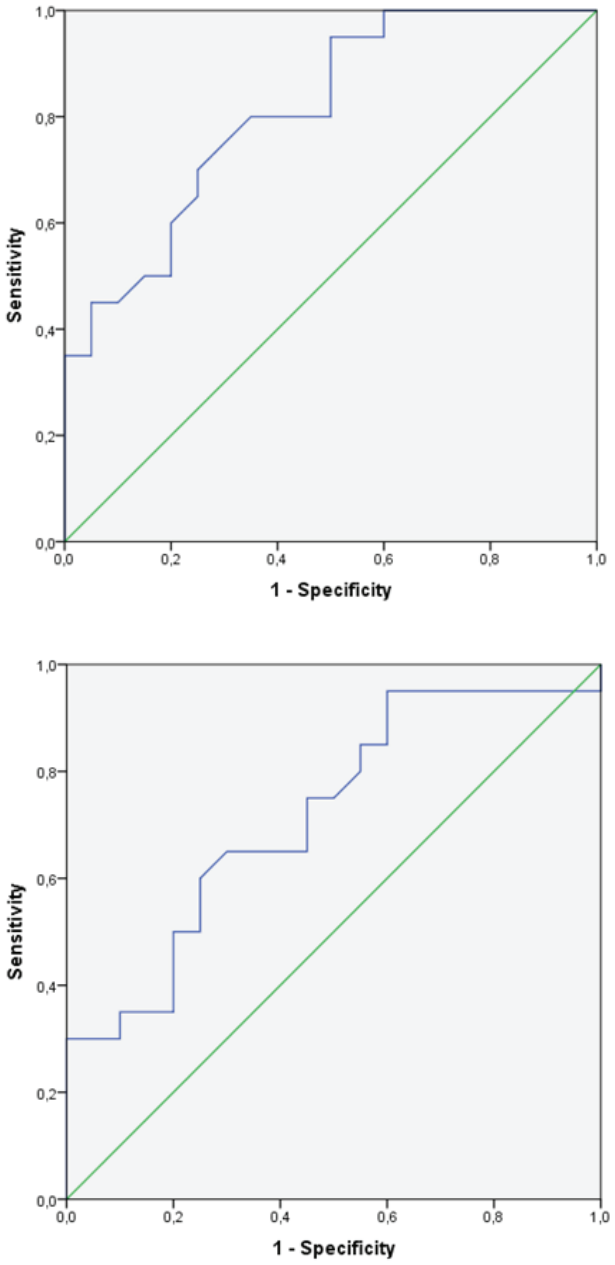

Figure 1. (A) ROC curve for vitamin D levels; (B) ROC curve for VDR expression.

The ROC curve in **Figure 1** shows the best cut-off value for vitamin D levels of 239.25 pg/mL (sensitivity 70% and specificity 75%) and VDR expression 2.23 ng/mL (sensitivity 60% and specificity 75%).

Table3. Effect of vitamin D levels and VDR expression on cervical cancer incidence

		Group		*P*	OR (95% CI)
		Normal	Cervical Cancer		
		N %	N %		
Vit D	low	6(30.0)	15(75.0)	0.04	2.71
	high	14(70.0)	5(25.0)		(1.22-6.04)
VDR	low	15(75.0)	8(40.0)	0.03	2.03
	high	5(25.0)	12(60.0)		1.07-3.84

Table 3 shows that low vitamin D levels increase the risk of cervical cancer incidence by 2.7 times greater and an increase in VDR expression increases the risk of cervical cancer incidence by 2 times greater.

In this study, the age between the two groups of subjects was significantly different, where the age of the cervical cancer group was significantly older of 47 years compared to the normal group of 37 years. The multivariate analysis by including the three variables (age, vitamin D level, and VDR) on the cervical cancer incidence can be seen in **Table 4**.

Table 4. Multivariate analysis of vitamin D level, VDR, and age on the incidence of cervical cancer

	P	OR	95% CI	
			Lower	Upper
Vitamin D	0.027	6.049	1.229	29.774
Age	0.008	11.875	1.891	75.576
Constant	0.01	0.179		

Multivariate analysis using logistic regression equation model I and model II. In the first model, the sig value of the VDR expression is > .05, so we omit it from the equation and move on to the next equation model.

The last equation model shows that vitamin D is a variable with a greater influence on the incidence of cervical cancer than age, where low vitamin D levels are of 6 times greater risk of cervical cancer, while premenopausal age increases the risk of cervical cancer by 11.8 times.

DISCUSSION

The mean vitamin D 1.25 (OH)2 levels in the cervical cancer group were significantly lower than in the normal group (*P*=.001), whereas the expression of vitamin D receptors in the cervical cancer group was significantly higher than the normal group (*P*=.018).

The study conducted by Ozgu et al. [5] determining the association between HPV-DNA infection and cervical intraepithelial neoplasia with vitamin D deficiency states that the average difference in vitamin 25 (OH) D3 levels between the positive HPV-DNA groups is 8.0857 IU/ml and the control groups 11.4720 IU/ml, statistically significant (*P*=.009). According to the results of this study, with the proven anti-inflammatory function of vitamin D, lack of molecules and metabolites of vitamin D can be a possible reason for persistent HPV-DNA and associated cervical intraepithelial neoplasia.

Gynecological cancer often occurs with high morbidity and mortality throughout the world. However, the association between gynecologic cancer and serum vitamin D is still controversial. Yan et al. [6] conducted a meta-analysis to evaluate the relationship between serum vitamin D deficiency and the occurrence of benign and malignant gynecological tumors. The study mentioned the occurrence of vitamin D deficiency in the case and control groups of respectively 52.36% and 48.70%; in women with benign and malignant reproductive tumors of 55.57% and 50.59%, respectively. Although no conclusive relationship was found between vitamin D deficiency and female reproductive tumors (OR 1.05, 95% CI 0.85-1.31), vitamin D deficiency can be a risk factor for gynecological malignancies (OR 1.17, 95% CI 1.02-1.33). Yan concluded that vitamin D deficiency seems to be a common problem in women, and there may be an urgent need to increase vitamin D levels. Furthermore, vitamin D deficiency may be a risk factor that cannot be excluded from gynecological malignancies.

A Japanese case-control study in 2010 was able to show a reduced risk of cervical cancer with increasing vitamin D intake [7]. Wang et al. [8] showed that vitamin D decreased the expression of cervical cancer oncogene, HCCR-1, and increased expression of p21, leading to the termination of the cell cycle in G1. Avila et al. [9] showed the inhibitory effect of calcitriol on human potassium à-go-go-1 channels (EAG1), which showed oncogenic properties.

VDR is expressed in large amounts of tumor tissues, showing that the receptors affect the etiology of cancer [10]. VDR polymorphisms have been shown to alter the complex activity of vitamin D-VDR. Their correlation with different types of cancer has been investigated to produce heterogeneous results. The polymorphisms most commonly associated with tumorigenesis are Bsm1, Fok1, Taq1, and Apa1 [11]. In addition, polymorphisms that occur in the single nucleotide vitamin D receptor (VDR) or referred to as single-nucleotide polymorphisms (SNPs) will affect the activity and, ultimately, the risk of cancer [12]. Reichrath et al. [13] analyzed the immunohistochemical expression of 1.25-dihydroxy-vitamin D3 receptor (VDR) in normal cervical tissue and in cervical carcinoma revealing that VDR experienced up-regulation of protein levels in cervical carcinoma compared to normal cervical tissue induced not exclusively by changes in epithelial differentiation or proliferation, but by different unknown mechanisms.

VDR and anabolic and catabolic vitamin D hydroxylases are expressed in a higher proportion of cervical carcinomas compared with the healthy cervical tissue. Besides, EAG1 is overexpressed in cervical cancer and increased expression of the EAG1 gene by the etiological factors of cervical cancer, that is, estrogens and HPV oncogenes. The incubation of CYP27B1-transfected SiHa cells with calcitriol precursor 25OHD3 causes an increase in endogenous calcitriol production and is similar to the inhibitory effect of calcitriol exogenously given on EAG1 gene expression. Such calcitriol production is sufficient to induce the expression of 24-hydroxylase mRNA, the vitamin D responsive gene. Because SiHa cell proliferation is not inhibited by calcitriol, VDR can be a new target for cervical prevention and treatment [14].

Vitamin D can act directly on cell proliferation and differentiation through the cell core vitamin D receptor (VDR) and regulate gene expression, including several proteins involved in the phosphorylation of retinoblastoma proteins that control the entry of cells into the cell cycle. Also, vitamin D can act indirectly by modifying the expression of growth factors as well as the immune system [15].

In conclusion, vitamin D and its association with the development and prevention of cancer must be a specific area to be investigated in depth. Revealing the possible preventive effects of this molecule on carcinogenesis allows us to have weapons, naturally occurred in cancer therapy and prevention. Further studies need to focus on VDR and its effects on cervical cancer. The relationship between vitamin D and VDR with gynecological cancer should be the focus of future studies that can lead to a better understanding of the molecular pathway. The limitation of this study is the sample of only 40 while the analysis uses the binary logistic regression method with 3 independent variables of age, vitamin D, and VDR.

CONCLUSIONS

The increased expression of VDR and decreased vitamin D 1.25 (OH)2 levels in cervical cancer women compared to normal women were obtained. Low vitamin D levels increase the risk of cervical cancer incidence by 2.7 times greater and an increase in VDR expression increases the risk of cervical cancer incidence by 2 times greater.

DECLARATIONS

Competing of Interest

The authors declare no potential conflicts of interest.

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