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The Comparison of 25-Hydroxyvitamin D3 between Patients With and Without Cervical Cancer

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Abstract

BACKGROUND: Vitamin D was currently believed to have effects on numerous cancer pathogenic processes.

AIM: This study was to assess the correlation of Vitamin D serum level in women with carcinoma cervix and also evaluate the effect of carcinoma cervix on the Vitamin D serum.

METHODS: This was an observational with a cross-sectional study. Participants were women with cervical cancer who have not received any treatment, presented to the Oncology Clinic of the Obstetrics and Gynecology Department of Dr. Hasan Sadikin General Hospital, and women without cervical cancer. The level of Vitamin D3 was analyzed in the Serology Laboratory of Clinical Pathology Department of Dr. Hasan Sadikin General Hospital.

RESULTS: There were 113 participants consisted of 58 women with cervical cancer and 55 healthy women. Mean levels of Vitamin D3 were significantly lower in cervical cancer group than non-cervical cancer group (26.74 ± 13.166 vs. 32.16±14.86, p = 0.022).

CONCLUSION: The level of Vitamin D3 was found to be significantly higher in the non-cervical cancer group than cervical cancer group.

Introduction

Cervical cancer is considered as a global burden due to its high prevalence and mortality. Numerous studies have contributed to better management of cervical cancer patients, however, the prevalence and mortality remain high. This urges the need for better prevention measures, rather than just focusing on patient management [1], [2]. Vitamin D or 25-hydroxyvitamin D3 has been known for its effects on important physiological processes on calcium and phosphate homeostasis, along with bone mineralization and osteogenesis, however, now studies have discovered that Vitamin D also has various extraskeletal effects related to the incidence and prognosis of many diseases including cardiovascular diseases, diabetes, and cancer. Vitamin D is currently believed to have effects on carcinogenesis, involving inflammation, apoptosis, cell proliferation and differentiation, angiogenesis, cancer invasiveness, and metastasis [3], [4], [5], [6]. Vitamin D increases apoptosis, reduces cell proliferation, and induces the expression of growth inhibitory molecules.

Vitamin D also reduces angiogenesis through molecular processes and reduces cancer invasiveness and metastasis through the regulation of cancer progressivity molecules expressions [4], [7], [8]. This makes the Vitamin D as an important and highly potential substance for cervical cancer's primary and secondary prevention. On top of that, patients with cervical cancer are believed to have a lower level of Vitamin D compared to healthy individuals. However, the data on Vitamin D in relation to cervical cancer are still scarce and inconclusive. Many studies related to Vitamin D to other types of cancer, such as colorectal, prostate, and breast cancer [9], [10], [11]. This study was determined to compare the level of Vitamin D3 between patients with cervical cancer and healthy women.

Methods

Design

This observational analytic study with cross-sectional design was conducted in the Oncology Clinic of the Obstetrics and Gynecology Department and the

Serology Laboratory of Clinical Pathology Department in Dr. Hasan Sadikin General Hospital in December 2019–February 2020. This study was approved by the Research Ethics Committee of the Faculty of Medicine, Padjadjaran University/Dr. Hasan Sadikin Hospital (LB.02.01/X.6.5/340/2019). The subjects were separated into two groups (cervical cancer and non-cervical cancer) and then compared the levels of Vitamin D between two groups.

The selection of research subjects begins with determining the population and determining inclusion and exclusion criteria. This study's population was women of reproductive age with a range of 15–49 years who go to the Oncology Polyclinic of Hasan Sadikin Hospital, Bandung. The cervical cancer group was all women with new cases of cervical cancer who met the inclusion and exclusion criteria. Cervical cancer group patients have been diagnosed with cervical biopsy examination and have not started therapy. All research subjects who met the inclusion and exclusion criteria will have a gynecological examination, laboratory examinations (Vitamin D levels), thorax radiology, CT scan, ultrasonography, and MRI serum Vitamin D levels to determine the stage of cervical cancer. The examination of 25-hydroxyvitamin D3 levels was carried out in the clinical serology Laboratory of Clinical Pathology, Hasan Sadikin Hospital, Bandung. The procedure for checking the levels of 25-hydroxyvitamin D3 is by taking 3 cc of venous blood into the tube and leaving it for 30 min. Then, the samples were centrifuged at 3000 rpm for 15 min, separating two aliquots of 0.5–1 mL each, and store samples at –20°C before examination with the electrochemiluminescence immunoassay method.

Participants and recruitment

Subjects were recruited by total sampling and selected using inclusion and exclusion criteria. All women with cervical cancer who fit the inclusion and did not fit the exclusion criteria were included into cervical cancer group and women with no cervical cancer who were willing to join the study were included in the non-cervical cancer group. The inclusion criteria for cervical cancer group were women with a new case of primary of cervical cancer, subjects were patients of oncology clinic but had not started therapy yet, subjects were willing to be participants of this study. The inclusion criteria for non-cervical cancer group were subject had no other type of cancer, and subjects willing to be participants of this study. The exclusion criteria were women who had chronic diseases, other cancer, or under treatment medication.

The sample size was determined using formula for numerical categorical analysis study sampling formula:

$$n_1 = n_2 = 2\left(\frac{Z_\alpha + Z_\beta S}{X_1 + X_2}\right)^2$$

$$n_1 = n_2 = 2\left(\frac{1.96 + 1.64 \times 1}{1}\right)^2$$

$$n = 2\ (13.03) = 26.03 \approx 27$$

Therefore, the total of minimum sample for this study was 27 subjects each category, then added by 10% of possible subject exclusion resulting in the minimum sample of 27 + 2,7 = 29.7 each category or around 30+30=60 subjects.

Variables and measurement

Independent variable of this study was patients with cervical cancer, normal healthy women (non-cervical cancer). The dependent variable in this study was the level of 25-hydroxyvitamin D3. The confounding variables were age and body mass index (BMI). Level of 25-hydroxy-vitamin D3 was measured by enzyme-linked immunosorbent assay (ELISA) kits in the serology laboratory of clinical pathology department of Dr. Hasan Sadikin General Hospital.

Statistical analysis

Data of this study were analyzed using SPSS 24.0 for Windows. Bivariate analysis was done using various methods. Normality test was done using Shapiro–Wilk test. Unpaired t-test was done if the data were normally distributed and alternatively Mann–Whitney U-test if the data were not normally distributed. Categorical data were analyzed using Chi-square or alternatively exact Fisher and Kolmogorov–Smirnov if

Table 1: Subject characteristics

Variable	Groups		p-value
	Cervical cancer	Non-cervical cancer	
	n = 58	n = 55	
Age			0.930
Mean ± SD	47.07 ± 7.276	46.00 ± 5.136	
Median	47.00	44.00	
Range (min-max)	30.00–63.00	30.00–56.00	
Parity			0.07
Nulliparity	0 (0.0%)	10 (18.2%)	
Primiparity	9 (15.5%)	7 (12.7%)	
Multiparity	44 (75.9%)	36 (65.5%)	
Grande multiparity	5 (8.6%)	2 (3.63%)	
Menarche (year)			0.882
Mean ± SD	11.05 ± 1.330	11.03 ± 1.104	
Median	11.00	11.00	
Range (min-max)	9.00–14.00	8.00–13.00	
BMI			0.175
Mean ± SD	19.72 ± 4.397	21.22 ± 3.602	
Median	20.7	21.2	
Range (min-max)	10.30–27.4	11.10–33.70	
Marital status			0.001
Married	58 (100.0%)	45 (81.8%)	
Not married	0 (0.0%)	10 (18.2%)	
Age of first sexual intercourse			0.001
Mean ± SD	18.06 ± 2.882	18.29 ± 9.635	
Median	18.00	22.00	
Range (min-max)	13.00–24.00	16–30.00	
Number of sex partner			0.02
0	0 (0.0%)	10 (18.2%)	
1	51 (87.9%)	42 (76.3%)	
2	7 (12.1%)	3 (5.45%)	

p-values of the variable age were tested using Mann–Whitney, parity and number of sex were tested using Kolmogorov–Smirnov, menarche and age of first sexual intercourse were tested using Mann–Whitney, BMI was tested using unpaired t-test, marital status was tested using exact Fisher. The level of significance was $p < 0.05$

the conditions for Chi-square were not met. Variables were statistically significant if $p \leq 0.05$.

Results

The total of participants in this study was 113, with 58 women in the cervical cancer group and 55 in the non-cervical cancer group. No statistically significant differences were found in age, parity, BMI, and menarche, among the characteristic data between the groups, indicating that both groups were homogenous and feasible to continue the statistical test (Table 1). The 1st age of sexual intercourse ($p = 0.001$), marital status ($p = 0.001$), and the number of sexual partners ($p = 0.02$) were significantly difference between the groups (Table 1). The mean of Vitamin D3 levels was found 26.74 ± 13.166 in cervical cancer group and 32.16 ± 14.866 in non-cervical cancer group (Table 2). Vitamin D3 was found significantly lower in the cervical cancer women group compared to non-cervical cancer women group ($p = 0.22$).

Table 2: Levels of Vitamin D3 in both groups

Variable	Groups		p-value
	Cervical cancer	Non-cervical cancer	
	n = 58	n = 55	
Levels of Vitamin D3			0.022
Mean ± SD	26.74 ± 13.166	32.16 ± 14.866	
Median	23.50	27.87	
Range (min-max)	9.18–75.93	12.65–72.73	

p-values of the numerical data were tested using unpaired t-test. The level of significance was $p < 0.05$

Discussion

Human knowledge about the function of Vitamin D has developed in the past 20 years. Vitamin D plays a role in calcium and phosphate hemostasis and bone mineralization, and osteogenesis. At present, Vitamin D shows interesting extraskeletal effects because it is related to the incidence and prognosis of critical medical conditions such as cancer, cardiovascular disease, or diabetes [3], [4], [5], [12], [13], [14], [15], [16].

The levels of Vitamin D3 were found significantly lower in the cervical cancer group compared to non-cervical cancer group. It is related to the one of the theories that Vitamin D and its receptors play a role in the inflammation process. Inflammation in cancer plays a role in the process of angiogenesis, cell growth, invasion, metastasis, response to treatment, and immunity [17]. Vitamin D helps regulation in decreasing NF signaling, one of the essential factors in inflammation [8]. Apoptosis also regulated by Vitamin D and its receptors. In one study, it was proven that mice without Vitamin D receptors (VDR) were found to have delayed apoptosis in the mammary epithelium [18], [19]. A study by Hosono *et al*. in a population in Japan found that Vitamin D consumption provides a protective effect against invasive cervical carcinoma [14].

Vitamin D3 is metabolized in the liver to 25-hydroxyvitamin-D3 and then metabolized in the kidneys into its active form, 1,25-dihydroxyvitamin-D3 [4]. The end product of the Vitamin D hydroxylation process is calcitriol [21]. The calcitriol arrives at the target cell and binds to the VDR protein. The VDR will then undergo changes that eventually form Vitamin D response elements [22]. In the literature, there has been found an association between Vitamin D and cervical cancer. A study by Friedrich *et al*. found that the VDR protein was found elevated in cervical cancer [13]. The increased VDR could be due to a decrease in the result of the hydroxylation of Vitamin D. The same study also found that cervical cancer can target Vitamin D analog therapy that may have both therapeutic and preventive effects [13]. VDRs act as nucleus receptors for cells called genomic effects and affect non-nuclei as non-genomic receptors. The VDR is a transcription-dependent ligand factor that belongs to the steroid hormone and thyroid hormone superfamily genes [24].

Vitamin D and its analogs can also reduce the expression of telomerase reverse transcriptase, which is commonly found in cancer cells that contribute to immortal phenotypes in malignant cells [25]. VDRs were able to suppress the transcription of hypoxia inducible factor 1 alpha which encourages the expression of vascular endothelial growth factor, one of the main regulators of angiogenesis. In the same way, Vitamin D can also reduce interlekin-8 through downregulation by $NF_k\beta$ [26]. The results of this study were consistent with the existing theory, thereby Vitamin D deficiency is considered as one of the factors in the etiology that triggers cervical cancer. This study's limitations were that there was no dietary recall for foods containing Vitamin D and the length of exposure to sunlight. Furthermore, data on the average Vitamin D status in the Indonesian population are minimal, so there is no reference value for 25-hydroxyvitamin D3 levels for the average Indonesian. Researchers also did not compare Vitamin D levels in various types of cervical cancer in the cervical cancer group.

Conclusion

The level of Vitamin D3 was found to be significantly higher in the non-cervical cancer group than cervical cancer group.

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