

# Serum 25-Hydroxyvitamin D Level and Breast Cancer Risk in Egyptian Females

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Asian J Oncol 2022;8:76–80.

## Abstract

Vitamin D has potent antiproliferative, prodifferentiative, and immune-modulatory effects. Vitamin D deficiency has been suggested to be very prevalent and there is growing evidence for the association between vitamin D deficiency and risk of breast cancer. The aim of this study was to evaluate the association of serum 25-hydroxyvitamin D [25(OH)D] level with breast cancer risk among Egyptian women. The current study included 40 breast cancer cases and 40 healthy control women. Serum 25 (OH)D levels were measured using enzyme-linked immunosorbent assay for all women and together with other clinical factors were correlated to the risk of breast cancer. A total of 80 women including 40 breast cancer cases and 40 controls were included in this analysis. The clinical characteristics were well balanced with no significant difference between cases and controls regarding age, menopausal status, weight, height, body mass index, serum calcium, and phosphorus levels. The mean serum 25 (OH)D level in cases (12.11 ng/mL) was significantly lower than in controls (19.77 ng/mL). Ninety percent of cases had 25(OH)D deficiency (<20 ng/mL) compared with 57.5% of the controls. After adjustment for potentially confounding variables, women with vitamin D deficiency were associated with a high significant risk of breast cancer compared to women with sufficient vitamin D with OR of 6.99 (95% CI = 2.01–24.32,  $p = 0.002$ ). A significant association exists between vitamin D deficiency and the risk of breast cancer in Egyptian women.

## Keywords

- ▶ breast cancer
- ▶ risk
- ▶ vitamin D

## Introduction

Worldwide, breast cancer is the most frequently diagnosed cancer and the leading cause of cancer death among women. Globally, approximately 2.1 million new cases were diagnosed in 2018, accounting for approximately 25% of all new

cancer cases in women.<sup>1</sup> According to the Egyptian National Cancer Registry Program, breast cancer was the most common female cancer and constituted 32% of all cancer among women in 2015.<sup>2</sup>

Breast cancer is a multifactorial disease with several established risk factors including positive family history,

DOI <https://doi.org/10.1055/s-0042-1748494>.  
ISSN 2454-6798.

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old age, high breast tissue density, early menarche, late age at menopause, nulliparity, late age at first birth, and genetic risk factors such as BRCA mutations.<sup>3,4</sup> Other risk factors include regular alcohol consumption, obesity, radiation exposure, and smoking.<sup>3</sup>

Vitamin D is a fat soluble vitamin that is produced mainly (up to 90%) through the conversion of 7-dehydrocholesterol to pre-vitamin D3 (cholecalciferol) under the skin by solar ultraviolet-B (UVB) radiation exposure. Then, cholecalciferol undergoes the first hydroxylation in the liver to form 25-hydroxyvitamin D [25(OH)D] or calcidiol. A second hydroxylation takes place in the kidneys to form 1,25-dihydroxyvitamin D [1,25(OH)2D] or calcitriol, which is the biologically active form of vitamin D. The half-life of 1,25(OH)2D is only 4 to 6 hours and 1,000-fold less than the total 25(OH)D. So, serum vitamin D status is usually determined by measuring 25(OH)D that has a half-life of approximately 2 to 3 weeks.<sup>5</sup>

Vitamin D has both rapid non-genomic actions that include calcium absorption in the small intestine, bone mineralization, and maintenance of calcium homeostasis in the blood, and slow genomic actions that are exerted via vitamin D receptor which is a nuclear receptor protein that is active in virtually all tissues including both normal and cancerous breast tissues. Vitamin D has potent antiproliferative, prodifferentiative, and immune-modulatory effects as well as a role in DNA repair. These effects could be the reason that deficient vitamin D levels might lead to cancer development.<sup>6-8</sup>

Low levels of vitamin D are frequently found in our population due to darker skin pigmentation and reduced UVB exposure due to traditional and religious type of dress. Several studies have evaluated the relationship between low vitamin D levels and breast cancer risk with controversial results.<sup>9-13</sup> The aim of this study was to evaluate the correlation of serum 25-hydroxyvitamin D level with breast cancer risk among Egyptian women.

## Methods

### Study Subjects

A total of 40 cases with pathologically proven breast cancer and 40 apparently healthy women matched on age (controls) who were receiving standard medical check-ups at the women's clinic, Oncology department, Alexandria main university hospital during the period from January 2015 to January 2016 were recruited. The inclusion criteria included; (a) women between the ages of 20 and 60 years; (b) body mass index (BMI)  $\leq 40$  kg/m<sup>2</sup>; (c) Absence of chronic diseases that could affect vitamin D metabolism, including renal, hepatic, endocrine, or autoimmune disease. Exclusion criteria included: (a) male; (b) age  $<20$  or  $>60$  years; (c) BMI  $>40$  kg/m<sup>2</sup>; (d) presence of chronic diseases that could affect vitamin D metabolism including renal, hepatic, endocrine or autoimmune disease; (e) intake of medications that affect bone metabolism and vitamin supplements within the last 6 months. This study was approved by the Local Ethics Committee of the Alexandria Faculty of Medicine and all

study subjects were informed and signed a written informed consent before study enrollment.

### Sample Collection and Measurement of 25(OH)D

A sample of 3 mL venous blood was withdrawn from the breast cancer patients and the healthy controls under complete aseptic conditions. The blood was added to a plain tube without anticoagulant, left for 10 minutes at room temperature to clot, and then serum was separated and stored at  $-20^{\circ}\text{C}$  until the time of assay of 25(OH)D. Serum 25(OH)D levels were measured using an enzyme-linked immunosorbent assay kit supplied by DRG International Inc. Frauenbergstr.18, D-35039Marburg, Germany; following the manufacturer's instructions. Women with serum 25(OH)D levels  $<20$  ng/mL were considered as 25(OH)D deficient, while those with serum 25(OH)D levels<sup>3</sup>  $\geq 20$  ng/mL were considered as 25(OH)D sufficient.<sup>14</sup>

### Statistical Analysis

The analysis was done using SPSS software version 22 (<http://www.ibm.com>). Descriptive analyses (frequencies, means, and SDs) for clinical characteristics were performed. Chi-square analyses were used for comparisons of categorical variables and independent-samples *t*-test for comparisons of continuous variables. The association between 25(OH)D concentration and risk of breast cancer was assessed using logistic regression modeling to estimate odds ratios (ORs) and 95% confidence intervals (CIs). Multivariate logistic regression was adjusted for potentially confounding variables including age, BMI, menopausal status, serum calcium, and phosphorus levels. All analyses were two-sided, with *p*-values  $<0.05$  indicating significance.

## Results

Data from 80 women including 40 breast cancer cases and 40 controls were included in this analysis. As shown in ► **Table 1**, the clinical characteristics were well balanced with no significant difference between cases and controls regarding age, menopausal status, weight, height, BMI, serum calcium and phosphorus levels. The mean age of breast cancer cases was 48.33 years and 45.95 years for the control group. About 40% and 50% of the cases and controls were premenopausal, respectively. The mean weight was 82.1 kg for cases and 84.5 kg for controls. The mean height was approximately 164 cm for both groups. The mean BMI for cases was 30.6 kg/m<sup>2</sup> and 31.2 kg/m<sup>2</sup> for controls. The mean serum calcium level was 9.3 mg/dL and 9.4 mg/dL for cases and controls, respectively. The mean serum phosphorus level was 3.6 mg/dL for cases and 3.5 mg/dL for controls.

Highly significant difference (*p* = 0.000) was found between cases and controls with regard to serum 25(OH)D level with a mean value of 12.11 ng/mL in cases and 19.77 ng/mL in controls (► **Table 1**). Serum 25(OH)D deficiency ( $<20$  ng/mL) was seen in 36 cases (90%), while 23 women of the controls (57.5%) had vitamin D deficiency. Using logistic regression model adjusted for potentially confounding variables (► **Table 2**), women with vitamin D deficiency were

**Table 1** Clinical characteristics of 80 Egyptian women (cases + control)

Variables	Cases (n = 40) Mean ± SD	Controls (n = 40) Mean ± SD	p
Age (y)	48.33 ± 7.31	45.95 ± 5.90	0.114
Menopausal status			
Pre- n (%)	17 (42.5%)	19 (47.5%)	0.653
Post- n (%)	23 (57.5%)	21 (52.5%)	
Weight (kg)	82.08 ± 9.28	84.48 ± 8.08	0.221
Height (cm)	163.78 ± 5.51	164.52 ± 4.61	0.511
BMI (kg/m <sup>2</sup> )	30.63 ± 3.35	31.24 ± 3.01	0.391
Calcium level (mg/dL)	9.26 ± 0.85	9.36 ± 0.87	0.613
Phosphorus level (mg/dL)	3.55 ± 0.53	3.51 ± 0.54	0.787
Vitamin D level (ng/mL)	12.11 ± 5.48	19.77 ± 7.73	0.000
Premenopausal Vit D (ng/mL)	13.58 ± 4.78	18.77 ± 8.73	0.037
Postmenopausal Vit D (ng/mL)	11.03 ± 5.81	20.68 ± 6.78	0.000
Vitamin D Category			
Deficient n (%)	36 (90%)	23 (57.5%)	0.001
Sufficient n (%)	4 (10%)	17 (42.5%)	

Abbreviations: BMI, body mass index; n, number; SD, standard deviation; Vit D, vitamin D.

**Table 2** Crude and adjusted ORs (95% CIs) for the association between vitamin D status and risk of breast cancer

	SE	p	OR	95% CI for OR	
				Lower	Upper
Adjusted vit D deficient for whole cohort	0.636	0.002	6.997	2.013	24.318
Crude vit D deficient for					
Whole cohort	0.617	0.002	6.652	1.987	22.271
Premenopausal	0.794	0.207	2.722	0.574	12.914
Postmenopausal	1.112	0.007	20.000	2.262	176.806

Abbreviations: CI, confidence interval; OR, odds ratio; SE, standard error; vit D, vitamin D.

Note: Crude = logistic regression models with no adjustments; Adjusted = logistic regression models adjusted for age, BMI, menopausal status, serum calcium, and phosphorus levels.

associated with a high significant risk of breast cancer compared to women with sufficient vitamin D with OR of 6.99 (95% CI = 2.01–24.32,  $p = 0.002$ ).

In exploratory subgroup analysis, despite the mean serum 25(OH)D level in premenopausal cases (13.58 ng/mL) was significantly lower than in pre-menopausal controls (18.77 ng/mL), this difference in pre-menopausal vitamin D deficiency was associated with a non-significant risk ( $p = 0.207$ ) of breast cancer. On the other hand, the mean serum 25(OH)D level in postmenopausal cases (11.03 ng/mL) was significantly lower than in postmenopausal controls (20.68 ng/mL) and postmenopausal vitamin D deficiency was associated with a highly significant ( $p = 0.007$ ) risk of breast cancer.

## Discussion

Vitamin D deficiency has become a major health concern after the discovery of great extent of populations suffering

from its various health consequences. Reports showed that the general population are not getting sufficient amount of vitamin D due to the current lifestyle and environmental factors that limit sunlight exposure. Further, melanin is an efficient filter of solar UVB radiation leading to more prevalence of vitamin D deficiency among darker skin population.<sup>15,16</sup> Despite the sunny weather in Egypt, vitamin D deficiency is very prevalent among Egyptian women due to multiple contributing factors including darker pigmented skin, covering most of the skin for cultural and religious reasons (being a Muslim community), avoiding performing activity in sunny areas, and use of sun blocks as well as the dietary regimen.

The present study showed that most breast cancer participants were vitamin D deficient (90%) with a significant difference in the mean serum level of 25(OH)D found between breast cancer cases and controls. The risk of developing breast cancer was seven times higher among vitamin D

deficient women. Our results are consistent with several studies that have demonstrated similar findings. The association between circulating concentrations of 25(OH)D and breast cancer risk in Saudi Arabian women was evaluated in a case-control study. The authors reported that the mean age was 47.8 years and a mean BMI was in 30 kg/m<sup>2</sup>. Breast cancer cases had significantly lower mean serum concentrations of 25(OH)D than did controls and an inverse association exists between serum 25(OH)D concentrations and breast cancer risk in Saudi Arabian women.<sup>10</sup> Comparable to our finding, in a meta-analysis of 2,877 Saudi Arabian women the mean serum 25(OH)D concentration was 13.1 ng/mL.<sup>17</sup> Abdelgawad et al<sup>9</sup> reported that serum level of 25(OH)D in the breast cancer group was significantly lower than in the control group. The same was also reported by Imtiaz et al<sup>18</sup> who found that the majority (>90%) of breast cancer patients had vitamin D deficiency and the mean serum vitamin D level in the breast cancer patients was significantly ( $p < 0.001$ ) lower than in the control group.

Also similar to our results, in a population-based multi-case control Spanish study, the authors found a clear protective effect between 25(OH)D levels and breast cancer risk, with a significant dose-response trend.<sup>19</sup> In an Indian study, the majority of the patients with breast cancer were suffering from vitamin D deficiency and a significant association was found between low serum 25(OH)D levels <20 ng/mL and the risk of breast cancer.<sup>20</sup> Similarly in a meta-analysis of 30 prospective studies by Kim and Je<sup>13</sup> showed an association of high vitamin D status with low breast cancer risk.

Vitamin D has been suggested to inhibit the growth of breast cancer cells by inducing cell cycle arrest and stimulating apoptosis, as well as inhibition of invasion, metastasis, and tumor angiogenesis. Additionally, vitamin D may regulate the production of estrogen and progesterone from ovarian tissue and downregulate the expression of estrogen receptor  $\alpha$  and thereby attenuate estrogen signaling in breast cancer cells including the proliferative stimulus provided by estrogen.<sup>6-8</sup> Further, vitamin D decreases the expression of aromatase enzyme that catalyzes estrogen synthesis selectively in breast cancer cells and in the mammary adipose tissue by a direct repression of aromatase transcription and an indirect effect due to the reduction in the levels and biologically active prostaglandin E2. Finally, it has been reported that vitamin D has an important role in both innate and adaptive immune system.<sup>21</sup>

The results of the current study showed significant vitamin D deficiency in both pre- and postmenopausal breast cancer cases than in controls. However, this vitamin D deficiency was associated with risk of breast cancer only in postmenopausal women. Variation in the association between 25(OH)D and breast cancer risk by menopausal status, may potentially be due to competitive binding of vitamin D and estrogen at lower levels of circulating 25(OH)D.<sup>22</sup>

Consistently, Bertone-Johnson et al<sup>23</sup> reported in a case-control study that despite breast cancer cases had a lower mean 25(OH)D level than controls ( $p = 0.01$ ), high levels of vitamin D was associated with a non-significant lower risk of breast cancer. However, the association was stronger in

postmenopausal women<sup>3</sup> of 60 years. In a German study, Abbas et al<sup>11</sup> reported a strong protective effect for postmenopausal breast cancer through a better vitamin D supply as characterized by serum 25(OH)D measurement, with a stronger inverse association in women with low serum 25(OH)D concentrations. In a clinic-based case-control study, Janowsky et al<sup>12</sup> reported that there were significant mean differences in vitamin D levels between breast cancer cases and controls with a protective effect of vitamin D for breast cancer in White women and the association was stronger in women above the median age of 54 than in younger women.

This study has limitations including the small sample size, lack of information about solar UVB exposure, use of sun blocks, detailed dietary intake, and the effect of other well-established risk factors was not evaluated. Additionally, serum 25(OH)D levels were measured by a single blood sample collected at one time point to define vitamin D status and the lack of biosamples before breast cancer diagnosis. However, there is evidence that indicates that serum 25(OH)D level remains relatively stable over time and single vitamin D blood sample may be representative of long-term vitamin D status.<sup>24</sup> In addition, our cases were newly diagnosed and properly matched with controls. Furthermore, our results were adjusted for potential confounder including BMI and age and menopausal status and serum calcium levels, providing accurate risk estimates.

In conclusion, this case-control study showed that vitamin D deficiency in women is associated with a greater risk of breast cancer. Serum vitamin D status in women should be routinely assessed. In addition, casual sun exposure and possibly dietary supplementation are recommended to correct vitamin D deficiency among women.

#### Funding

None.

#### Conflict of Interest

None declared.

#### Acknowledgment

None.

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