Research Article

Association Between Vitamin D Status and Breast Cancer Among Iraqi Women: A Case–Control Study

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Abstract

Aim: To investigate the association between serum 25-hydroxyvitamin D [25 (OH) D] levels and breast cancer in females living in Najaf, Iraq.

Methods: A case-control study was carried out, consisting of 116 women (56 breast cancer patients and 60 controls with no cancer diagnosis). The 25(OH)D level was determined and categorized as low (below 12 ng/mL), insufficient (12–<20 ng/mL), and adequate (20 ng/mL or higher). Multivariable logistic regression was employed to assess the association between vitamin D with breast cancer, adjusting for age and body mass index (BMI).

Results : Mean serum 25(OH)D concentration was substantially lower among cases than controls (9.46 ± 5.85 vs. 23.42 ± 12.31 ng/mL). Vitamin D deficiency was observed in 78.6% of cases compared with 16.7% of controls, whereas adequate concentrations were observed in 3.6% and 46.7%, respectively. The distribution of vitamin D categories differed significantly between groups ($\chi^2(2) = 48.36$, $p < 0.001$; Cramér's V = 0.646). Compared with adequate vitamin D status, deficiency was associated with markedly higher odds of breast cancer (adjusted OR = 56.91, 95% CI: 10.75–301.19, $p < 0.001$), while generally inadequate status was also significantly associated with higher odds (adjusted OR = 11.10, 95% CI: 1.90–64.88, $p = 0.0075$).

Conclusion: The association between low levels of 25(OH)D and breast cancer was strong in this study population and the association remained statistically significant following adjustment for age and BMI. Further, larger studies are needed to elucidate the time course and possible associated with vitamin D and the development of breast cancer.

Keywords: Breast cancer, vitamin D, deficiency, women's health, Iraq.

Introduction

Breast cancer is the most widespread malignancy among women worldwide and ranks as one of the leading causes of cancer-induced mortality internationally. In 2024, it was projected that 2.4 million women would be diagnosed with breast cancer, with 694,000 fatalities attributed to the illness. A significant challenge that needs to be addressed is breast cancer, the most prevalent cancer among women in 164 out of 186 nations. Despite the advances made in early diagnosis and treatment of breast cancer, its burden remains and it is important to explore factors that may be modifiable in terms of breast cancer development [1,2].

Besides the known associated factors of age, reproductive history, family history of breast cancer and hormonal factors, there has been a growing interest in potentially modifiable dietary and metabolic factors [3]. Of particular interest has been vitamin D. While vitamin D is known for its function in calcium metabolism and bone health, it also has been involved in multiple cellular functions such as proliferation, differentiation and apoptosis; these functions are mediated, in part, through vitamin D signaling pathways [4]. The biological functions serve as the foundation to study vitamin D status and possible links with breast cancer.

The relationship between breast cancer and vitamin D status remains unclear. Some observational studies have found an inverse link between the circulating 25-hydroxyvitamin D [25(OH)D] and breast cancer risk, albeit with variable results among populations and subgroups [5]. The results, however, have not been consistent across prospective cohort studies and randomized controlled trials, and reverse causation and residual confounding are important factors to consider. Differences between studies may reflect variations in study design, population characteristics, vitamin D measurement, and other potential confounding factors [6].

It is very common in the Middle East and North African region for vitamin D to be deficient, despite the high level of sunshine. There is some recent evidence that there may be lifestyle and cultural factors that cause low vitamin D status in the region, such as limited sun exposure and clothing to minimize skin exposure [7]. However, dietary

factors may also play a role, especially if vitamin D foods are not eaten frequently. In a recent study in Baghdad, lab-confirmed vitamin D deficiency was reported to be high especially among Iraqi women [8]. A recent Iraqi study has also reported lower serum 25-hydroxyvitamin D concentrations among women with breast cancer compared with healthy controls [9].
However, evidence from other Iraqi populations remains limited. Thus, this case–control study aimed to investigate the association between serum 25-hydroxyvitamin D [25(OH)D] level and breast cancer among women in Najaf, Iraq.

Methods

Study Design: The objective of this retrospective case-control study was to investigate the association between breast cancer and serum 25-hydroxyvitamin D [25(OH)D] levels in women residing in Najaf, Iraq. Women who had been diagnosed with breast cancer and had their diagnosis confirmed by histological examination were classified as cancer cases, while women who had never been diagnosed with cancer were recruited as controls.

Study Setting and Participants: This study used secondary data from the medical and laboratory records of women who presented to Al-Sadr Medical City in Najaf, Iraq, as a referral hospital for women diagnosed with breast cancer. The case group included 56 breast cancer patients diagnosed by biopsy, and the control group included 60 patients who had no history of breast cancer in the clinic. Demographic and clinical data (age, anthropometric data, smoking history, blood 25-hydroxyvitamin D [25(OH)D] level) was retrieved from the study documentation randomly. The participants' ages varied from 19 to 67 years. The experimental group comprised of breast cancer diagnoses women, and the control group comprised women with no breast cancer history.
The inclusion criteria were female participants fulfilling the study criteria and with available information that matched the study variables, specifically the serum 25(OH)D level. Those with other malignancies, pregnant women, lactating women, women taking vitamin D or calcium for the three months before the assessment, and chronic liver or kidney disease were excluded.

Data Collection: Data were collected from March to June 2025 using structured data collection forms and by reviewing participants' medical records randomly. Collected variables were age, height, weight, body mass index (BMI), smoking history and serum 25-hydroxyvitamin D [25(OH)D] levels. Relevant clinical information for participants in the breast cancer group was obtained from their medical records.

Laboratory Analysis: Serum vitamin D measurements were obtained from the existing medical and laboratory records. During the clinical assessment, venous blood samples were routinely drawn from patients at the hospital laboratory, according to the hospital laboratory protocol. After the blood is collected, the blood was centrifuged and the samples were kept in the freezer at −20 °C until analysis. The 25-hydroxyvitamin D [25(OH)D] was measured by a standardized chemiluminescence immunoassay as per the manufacturer's instructions. Vitamin D levels were measured in ng/mL. The available vitamin D results obtained from these records were retrieved in the present study.

Variable Definitions : Serum 25-hydroxyvitamin D [25(OH)D] concentrations were analyzed as a continuous variable and categorized according to the reference values described by the National Institutes of Health Office of Dietary Supplements (NIH ODS) [10]. Vitamin D concentrations of ≥20 ng/mL were considered adequate for most individuals, whereas concentrations of 12–<20 ng/mL were considered generally inadequate. Concentrations below 12 ng/mL were considered to indicate vitamin D deficiency.
Breast cancer status (case/control) was the primary outcome variable. The factors which were considered in the study were age, BMI, smoking status and contraceptive usage (intrauterine device (IUD) use). These variables were considered as potential factors associated with serum vitamin D concentrations and breast cancer status.

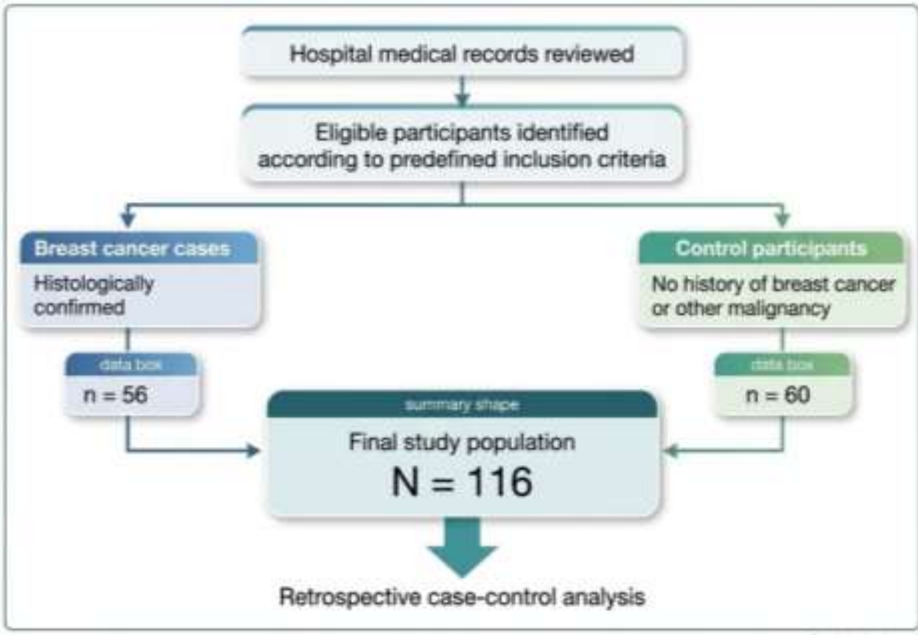

Figure 1: Study population selection diagram

Statistical analysis: Data were analysed using SPSS version 26, and continuous variables were presented as mean ± SD, while categorical variables were presented as number and percentage. The Mann–Whitney U test was used to make comparisons between the case and control groups. The chi-square test was used to analyze the categorical variables. To test the relationship between breast cancer and vitamin D in the presence of other variables such as age and BMI, logistic regression was used. Odds Ratios (ORs) and 95% Confidence Intervals (CIs) were presented. P values < 0.05 were considered statistically significant.

Ethical Considerations: The study was approved by the Central Committee of Ethics at Al-Sadr Medical City, Najaf, Iraq (Approval No. 102, dated February 9, 2025). Personal identifiers were removed from the data and only members of the research team with permission had access to the data, thereby protecting patient confidentiality and privacy. All participants gave informed consent.

Results

Study Population

The investigation included 116 women, 56 of whom were breast cancer cases and 60 of whom were controls. The mean age of cases was greater than that of controls (49.61 ± 11.37 vs. 37.40 ± 13.90 years, respectively). Similarly, the mean body mass index (BMI) was slightly higher among cases than controls (29.23 ± 5.46 vs. 27.89 ± 5.89 kg/m², respectively). Smoking was uncommon in both groups, being reported by 3.6% of cases and 3.3% of controls. IUD use was reported by 14.3% of cases and 6.7% of controls (Table 1).

Table 1. Baseline characteristics by group

Characteristic	Control (n = 60)	Case (n = 56)
Age, years (mean ± SD)	37.40 ± 13.90	49.61 ± 11.37
BMI, kg/m² (mean ± SD)	27.89 ± 5.89	29.23 ± 5.46
Smoking, n/N (%)	2/60 (3.3%)	2/56 (3.6%)
IUD use, n/N (%)	4/60 (6.7%)	8/56 (14.3%)

Table 1 presents the baseline characteristics of the study participants according to study group. The mean age was higher among participants in the case group (49.61 ± 11.37 years) than in the control group (37.40 ± 13.90 years). Similarly, the mean BMI was higher in the case group (29.23 ± 5.46 kg/m²) compared with the control group (27.89 ± 5.89 kg/m²). Smoking was uncommon in both groups, being reported in 3.6% of cases and 3.3% of controls. IUD use was reported by 14.3% of cases compared with 6.7% of controls. Overall, the case group tended to be older and had a somewhat higher mean BMI than the control group.

Serum Vitamin D Concentrations

Table 2 shows the serum 25-hydroxyvitamin D [25(OH)D] concentrations in the case and control groups. The mean serum vitamin D concentration was substantially lower in the case group (9.46 ± 5.85 ng/mL) than in the control group (23.42 ± 12.31 ng/mL). The median vitamin D concentration was also markedly lower among cases (8.24 ng/mL [IQR: 5.38–10.65]) compared with controls (19.35 ng/mL [IQR: 13.02–34.72]). The between-group difference in serum vitamin D concentrations was statistically significant (Mann–Whitney U = 432, $p < 0.001$), indicating that women with breast cancer had significantly lower serum vitamin D concentrations than controls.

Table 2. Serum Vitamin D by group

Group	n (with Vit D data)	Vitamin D, ng/mL (mean ± SD)	Vitamin D, ng/mL (median [IQR])
Control	60	23.42 ± 12.31	19.35 [13.02–34.72]
Case	56	9.46 ± 5.85	8.24 [5.38–10.65]

Prevalence of Vitamin D Deficiency

Table 3 presents the distribution of vitamin D status according to the reference categories used in this study. Vitamin D deficiency (<12 ng/mL) was substantially more common among cases than controls, occurring in 78.6% of cases compared with 16.7% of controls. The proportion of participants with generally inadequate vitamin D concentrations (12–<20 ng/mL) was 17.9% among cases and 36.7% among controls. In contrast, adequate vitamin D concentrations (≥20 ng/mL) were observed in only 3.6% of cases compared with 53.3% of controls. Overall, the distribution of vitamin D categories differed significantly between cases and controls ($\chi^2(2) = 48.36$, $p < 0.001$, Cramér's V = 0.646), indicating a strong association between vitamin D status and breast cancer case status.

Table 3. Distribution of vitamin D categories by group

Vitamin D category	Control (n = 60)	Case (n = 56)
Deficiency (<12 ng/mL)	10 (16.7%)	44 (78.6%)
Generally inadequate (12–<20 ng/mL)	22 (36.7%)	10 (17.9%)
Adequate (≥20 ng/mL)	28 (46.7%)	2 (3.6%)

Odds Ratios for Breast Cancer by Vitamin D Status

Multivariable logistic regression analysis, adjusted for age and BMI ,was performed to assess the association between vitamin D status and breast cancer (Table 4). Compared with women with adequate vitamin D concentrations (≥20 ng/mL), those with vitamin D deficiency (<12 ng/mL) had substantially higher odds of breast cancer (adjusted OR = 56.91, 95% CI: 10.75–301.19, $p < 0.001$). Moreover, women with a general level of vitamin D of 12–<20 ng/mL were at significantly higher risk for breast cancer than the women with an adequate level of vitamin D (adjusted OR = 11.10, 95% confidence interval: 1.90–64.88; $p = 0.0075$).

The covariates that were statistically significantly associated with breast cancer were age, which showed a positive association with breast cancer (adjusted OR = 1.06, 95% CI: 1.02–1.10, $p = 0.0076$) for every additional year. However, BMI was not significantly related to breast cancer in the adjusted model (BMI adjusted OR = 1.03, 95% CI: 0.93–1.15, $p = 0.527$).

The results suggest a significant association for the vitamin D deficiency, as well as for overall low levels of vitamin D, with breast cancer risk, relative to adequate vitamin D status. This association was strong among women who had vitamin D levels <12 ng/mL, with the women having about 57-fold higher odds of developing breast cancer than women with levels adequate for vitamin D. Odds of breast cancer were also about 11-fold higher for women with

levels from 12 to less than 20 ng/mL. In this model, age was an independent associated factor for breast cancer and BMI was not.
Overall, the results show a significant link between decreased vitamin D levels and increased risk of breast cancer, with the greatest association with women with vitamin D levels <12 ng/mL.

Table 4. Logistic regression of breast cancer on vitamin D categories (adjusted for age and BMI)

Predictor	Adjusted OR	95% CI	p-value
Deficiency (<12 ng/mL) vs Adequate (≥20 ng/mL)	56.91	10.75–301.19	<0.001
Generally inadequate (12–<20 ng/mL) vs Adequate (≥20 ng/mL)	11.10	1.90–64.88	0.0075
Age (per year)	1.06	1.02–1.10	0.0076
BMI (per kg/m²)	1.03	0.93–1.15	0.527

Discussion

Regarding the baseline characteristics results in Table 1, women with breast cancer were older than the control group and had a slightly higher mean BMI. The difference in age was particularly relevant because increasing age is an established factor associated with breast cancer and may also influence vitamin D status; therefore, age was considered an important potential confounder and was included in the adjusted logistic regression model. This finding is aligned because age and overweight have been recognized as established associated factors with breast cancer, as in a previous study [11].
Although the mean BMI was also higher among cases, the difference was relatively modest. Smoking was uncommon and showed a similar frequency in both groups, whereas IUD use was more frequently reported among cases than controls. Such differences should be taken into account when evaluating the relationship between serum vitamin D level and breast cancer. There has been conflicting evidence for the link between the use of levonorgestrel-releasing intrauterine device (LNG-IUD) and breast cancer, with some studies showing a possible small increase in risk, and others showing no statistically significant association [12]. Despite the methodological diversity and the possible confounding factors, however, the results of this study should be viewed with some caution. Thus, the increased prevalence of IUD use found among breast cancer cases in the current study should not be viewed as a causal relationship with breast cancer.
The serum vitamin D level of breast cancer women and the control group differed significantly in the current study, as illustrated in Table 2. The mean and median serum vitamin D levels of cases were significantly lower than those of controls, indicating that the vitamin D status of breast cancer women is generally poor. Our findings corroborate the findings of a recent study [13] that demonstrated that women with breast cancer had serum vitamin D concentrations that were lower than those of women without breast cancer. The study further presents the association between vitamin D insufficiency and breast cancer, as the prevalence of vitamin D insufficiency was higher among women with breast cancer than among controls.
In accordance with this, a significant number of women (78.6%) with breast cancer had severe Vitamin D deficiency (<12 ng/mL), as opposed to 16.7% of women in the control group. A vitamin D deficiency (<20 ng/mL) was detected in 96.4% of cases and 53.3%of controls. In contrast, 3.6% of cases had adequate vitamin D levels (≥20 ng/mL), while 53.3% of the control group did. The present study, which demonstrated the association between low vitamin D status and breast cancer, was corroborated by a previous study [14] that reported that breast cancer patients were significantly more likely to be vitamin D deficient than controls (85.7% vs. 55.8%, respectively). Additionally, a case-control study conducted in Sulaymaniyah, Iraq, demonstrated that the mean serum vitamin D levels of breast cancer patients were significantly lower than those of healthy controls. A total of 66.1% of breast cancer patients had serum vitamin D levels below 20 ng/mL, compared to 43.1% of healthy controls. The study also revealed that low vitamin D levels are associated with an elevated risk of breast cancer (OR = 2.59, 95% CI: 1.24–5.38, p =.009). The association between low vitamin D levels and breast cancer in the present study is consistent with results of this study in another Iraqi population [15]. The strong overall association between breast cancer case status and vitamin D category ($\chi^2(2) = 48.36$, $p < 0.001$; Cramér's V = 0.646) was evident in the significant difference in the distribution of vitamin D

categories between cases and controls. This study's findings, as well as prior research (including evidence from a population in Iraq) indicate an association between breast cancer and low serum vitamin D levels.

The multivariable logistic regression model demonstrated a robust association between breast cancer and low serum vitamin D status. The strongest association was observed among women with vitamin D deficiency (<12 ng/mL), who had approximately 57-fold higher odds of breast cancer compared with women with adequate vitamin D concentrations (≥20 ng/mL) (adjusted OR = 56.91, 95% CI: 10.75–301.19, $p < 0.001$). This finding is consistent with a case–control study conducted among Egyptian women, in which vitamin D deficiency (<20 ng/mL) was associated with significantly higher odds of breast cancer after adjustment for potential confounding factors (OR = 6.99, 95% CI: 2.01–24.32, $p = 0.002$) [16]. However, the difference in the magnitude of the association may partly reflect differences in vitamin D classification of Egyptian study defined deficiency as <20 ng/mL, whereas the present study used <12 ng/mL for deficiency and 12–<20 ng/mL for generally inadequate status. Thus, the higher OR observed in the present study may partly reflect the more severe deficiency represented by the <12 ng/mL category; both studies demonstrated a significant association between vitamin D deficiency and breast cancer after multivariable adjustment.

For women with generally inadequate vitamin D levels (12–<20 ng/mL), the odds of breast cancer were also significantly higher, about 11 times higher than that of women with adequate vitamin D levels (adjusted OR = 11.10; 95% CI: 1.90–64.88; $p = 0.0075$). Consistent with the direction of our findings, recent studies reported a non-significant inverse association between non-deficient 25(OH)D concentrations (>20 ng/mL) and breast cancer incidence compared with deficient concentrations (≤20 ng/mL) (HR = 0.79, 95% CI: 0.61–1.02). However, a statistically significant and stronger inverse association was observed among Hispanic/Latina women (HR = 0.52, 95% CI: 0.29–0.93) [17].

These results further confirm an inverse relationship between vitamin D status and breast cancer. Interestingly, the relationship between vitamin D status and breast cancer was found to still be statistically significant when age and BMI were also controlled for, suggesting that these factors alone did not fully explain the relationship between vitamin D and breast cancer. Low vitamin D levels (<20 ng/mL) were also found to be significantly associated with breast cancer even after adjusting for age and BMI (AOR = 2.30, 95% CI: 1.10–4.86, $p = 0.03$), which further supports the association between low vitamin D status and breast cancer among Iraqis [15].

In the adjusted model, age remained independently associated with the breast-cancer case status: The odds of being a case increased by about 6% for each extra year of age (adjusted OR = 1.06, 95% CI: 1.02–1.10, $p = 0.0076$). This finding is in line with population-based evidence from Iraq of rising incidence of breast cancer in older age groups [18]. Likewise, a population-based study conducted in Saudi Arabia recently showed significant differences in breast cancer incidence with the highest level of incidence seen in older women [19].

On the other hand, the adjusted model did not demonstrate a significant association between BMI and breast cancer (adjusted OR = 1.03, 95% CI: 0.93–1.15, $p = 0.527$). In the present study, the observed association between vitamin D status and breast cancer was not independently explained by BMI, despite the fact that the case group had a slightly higher mean BMI than the control group. This discovery contrasts with a recent systematic review and meta-analysis, which indicated a general association between an elevated body mass index (BMI), particularly obesity, and an increased risk of breast cancer. This discrepancy may be partially accounted for by variations in population characteristics and sample size [20].

Biologically, the development of breast cancer may be influenced by vitamin D, which is mediated by the vitamin D receptor (VDR). The active form of vitamin D has the potential to regulate signaling pathways involved in tumor development, promote cellular differentiation and apoptosis, and inhibit cell proliferation and migration. Consequently, a biological environment that is conducive to the development of breast cancer may be exacerbated by an insufficient vitamin D status [21, 22].

Despite the fact that the current findings indicate a robust association between low serum vitamin D status and breast cancer, additional prospective studies that are well-designed are required to elucidate the temporal relationship and potential function of vitamin D in the development of breast cancer.

Limitations

This study has several limitations. The case–control design limits the ability to establish temporality or causality, and reverse causation cannot be excluded because vitamin D measurements in breast cancer cases were obtained after diagnosis. The hospital-based selection of controls and the substantial age difference between cases and controls may have introduced selection and residual confounding, despite adjustment for age and BMI. Data regarding menopausal status, sunlight exposure, dietary habits, vitamin D supplementation, and physical activity and socioeconomic status were not available. Vitamin D level could have been affected by seasonal variation, but they were measured during the same time of the year and the same months. The small number of breast cancer cases in the adequate-vitamin-D category resulted in less precise estimates and wide confidence intervals. Lastly, the results from the single-centre study and the small sample size might limit the generalisability of the conclusions. Thus, the associations observed should be interpreted with caution and verified in larger and multicentre studies.

Conclusion

This case-control study illustrated a robust association between breast cancer and low serum 25-hydroxyvitamin D [25(OH)D] levels in women residing in Najaf, Iraq. Breast cancer cases were significantly more likely to exhibit vitamin D deficiency, and both deficient and generally inadequate vitamin D concentrations were associated with significantly higher odds of breast cancer than adequate concentrations, even after adjusting for age and BMI. The potential relevance of vitamin D status in breast cancer is also supported by these findings, which underscore the necessity of conducting larger prospective studies to elucidate the temporal relationship and potential function of vitamin D in the development of breast cancer.

Acknowledgments

The authors would like to express their appreciation to the staff of Al-Sadr Medical City, Najaf, Iraq for helping them obtain medical records and laboratory data for this study. The research did not have any specific funding source in the public, commercial or not-for-profit sectors.

Conflicts of Interest (COI) Disclosure

The authors do not have any conflicts of interest to disclose**.**

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