Serum 25-hydroxyvitamin D Concentration and Breast Cancer Risk in Egyptian Women in South Egypt

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Abstract:

Purpose: to examine the relationship between circulating vitamin D level and risk of breast cancer.

Methods: This case-control study was conducted in the surgical oncology departments at Assiut University's South Egypt Cancer Institute from September 1st, 2019 to May 31st, 2020. The study enrolled 140 people (70 cases with newly diagnosed breast cancer and 70 age-matched controls from hospital visitors). The researcher obtained informed consent from the study participants. The questionnaire included inquiries about the following aspects: serum vitamin D level; data pertinent to demographic and clinical characteristics; parity; menstrual and contraceptive histories; dietary history; sun exposure; BMI; and waist-to-hip ratio were collected by face-to-face interview and compared between the two groups. Regarding the breast cancer cases, pathologic characteristics were evaluated against the vitamin D level.

Results: The mean age of breast cancer cases was 51.54 vs. 50.11±5.44 years in the control group. Remarkable proportions of breast cancer cases and controls were found to have a low serum level of vitamin D. The study revealed that 94.3% of cases have vitamin D levels less than 20 ng/mL versus 78.6% in the control group. The odds ratio of breast cancer associated with a vitamin D level 20 ng/ml was more than 5 times higher with levels 20 ng/ml after adjustment for age, BMI, sun exposure, family income, education status, and family history of breast cancer.

Conclusion: Because vitamin D levels were found to be associated with the risk of breast cancer in the current study, every effort should be made to improve women's health and socioeconomic status, particularly in less privileged communities such as rural upper Egypt.

Key words: breast cancer, vitamin D deficiency, dietary history.

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Introduction:

Breast cancer has surpassed lung cancer as the most commonly diagnosed cancer, with an estimated 2.3 million new cases worldwide. It is the fifth-leading cause of cancer mortality worldwide, with 685,000 deaths. Among women, breast cancer accounts for 1 in 4 cancer cases and 1 in 6 cancer deaths [1]. Breast cancer accounts for 33 percent of all female cancer cases in Egypt, with more than 22,000 new cases diagnosed each year and a forecasted number of approximately 46,000 cases in 2050, reported by the World Health Organization [2]. Numerous risk factors for breast cancer have been studied and well documented: (a) family history and genetics; (b) reproductive and hormonal factors; (c) proliferative benign breast pathology; and (d) lifestyle [3]. In addition to its role in calcium and bone homeostasis, vitamin D regulates many other cellular functions; the vitamin D receptor (VDR) is nearly universally expressed in nucleated cells. Approximately 3 percent of the human or mouse genome is controlled by 1,25-dihydroxyvitamin D, the active form of vitamin D [4]. Several observational studies examined the relationship between serum vitamin D levels and cancer; some studies suggest a link between vitamin D deficiency and

cancer, while others show an increased risk of certain cancers (pancreatic) [5]. A meta-analysis of prospective studies on the relationship between serum 25(OH)D levels and breast cancer risk found a significant inverse association in postmenopausal women but not in premenopausal women [6]. A recent study showed that vitamin D deficiency among healthy Egyptian females was highly prevalent in different age groups [7]. Optimizing that low levels of vitamin D are a risk for breast cancer, modifications and corrective actions could be taken to reduce the disease burden among Egyptian females.

Aim of the study: to evaluate the relationship between circulating vitamin D level and risk of breast cancer.

Patients and Methods:

Study Design

A case-control study conducted at the South Egypt Cancer Institute's surgical oncology inpatient department. The hospital ethics committee approved the study design and all participants signed written informed consent. Study procedures were carried out according to the Code of Ethics of the World Medical Association (Declaration of Helsinki). All informations and images were anonymized, and the privacy rights of the study participants were observed diligently.

Study Participants

The present study included 140 females: 70 newly diagnosed breast cancer patients and 70 healthy females as a control group. The study was conducted at South Egypt Cancer Institute hospital, Assiut university, through the period from September 1st, 2018, to May 31st, 2020,

The study participants were analyzed by the researcher regarding demographics, clinical characteristics, contraceptive, menstrual, obstetric, dietary, and sun exposure histories, and for comparing the findings of the two groups regarding vitamin D, BMI, and waist/hip ratio. Moreover, pathologic characteristics of the breast cancer and the relationship with vitamin D levels were evaluated in breast cancer patients.

The study included screened participants with newly diagnosed and confirmed breast cancer who were mentally competent to answer the questionnaire and willing to sign a consent form for enrolment.

Methodology:

Sample size

A pilot study was done on 30 patients (21% of the calculated sample size), to evaluate the feasibility of the questionnaire and test for sensitive questions. It resulted in: (1) No editing was done, but some questions needed further clarification. (2) According to the results of the pilot study, more than 60% of the patients have complete illiteracy (cannot read or write), and the decision was made to help them understand the questions by the interviewer. (3) Prior data (Imtiaz S et al., 2012) indicated that the probability of vitamin D deficiency among controls is 0.77 (30 ng/ml) [8]. If the true probability of exposure among cases is 0.956, we need to study 57 breast cancer patients and 57 control patients to reject the null hypothesis that the exposure rates for cases and controls are equal with a probability of 0.8. The type I error probability associated with this null hypothesis test is 0.05.

The sample size was adjusted to include 70 individuals from each group, a total of 140 females, assuming that the rate of non-response would be at least 20%. (Program to Calculate Power and Sample Size; PS Version 3.1.2).

Rate of Compliance

A total of 193 participants were enrolled in the study. 140 out of them were eligible to participate in the study and were interviewed by the researcher with a refusal rate of 27.5%.

Material and tools of the study:

The questionnaire

The researcher developed the questionnaire and used it as tool for collecting the data via using the WHO non-communicable diseases survey format (NCD STEP). The collected data of the study participants included the socio-demographic and clinical characteristics, contraceptive history, menstrual history, and parity; dietary history and sun exposure; serum vitamin D; BMI; and waist/hip ratio. On average, data collection and blood sampling took one hour for each participant. pathological characteristics were reported in breast cancer cases only.

Assessment of sun exposure and dietary history

Women were asked about beneficial sunray exposure as measured by frequency and total cumulative time. Whether "daily, weekly, monthly, in winter only, or rare," as per the recommendation, is 25 to 30 minutes of sun exposure in the morning (right after sunrise and before 8 a.m.) and evening (at the time of sunset) from about late March or early April to the end of September, most people should be able to get all the vitamin D they need from sunlight [9].

The calculation of the cumulative period of solar exposure per month (minutes) =

- Daily exposure = daily exposure time (minutes) multiplied by 30
- Weekly exposure = weekly exposure time (minutes) *4
- Monthly exposure = monthly exposure time (in minutes).
- Winter exposure = daily exposure (minutes) in winter * (4 * 30)

Supplement intake, type of supplement, and period if the answer is yes.

Dietary history included relevant foods rich in vitamin D. It included the frequency of meat intake (one portion measuring 150 g), the frequency of chicken intake (one portion measuring 150 g), the median fish intake (one portion measuring 150 g), the frequency of egg intake (as one egg), the frequency of cheese intake

(one portion measuring 150 g), and the number of cups of milk drunk (measuring 250 ml or L) per month in the diet.

Assessment of serum vitamin D level by ELISA

5 ml of peripheral blood were withdrawn from all cases and the control group under aseptic precautions into sterile tubes (EDTA vacuum collection tubes, Becton-Dickinson, NJ, USA). Samples were stored at -20c until kits were available for assessment of 25-hydroxy (25-OH) Vitamin D. The 25-hydroxy (25-OH) Vitamin D ELISA kit is intended for the quantitative determination of total 25-OH Vitamin D in human serum, with regard to Vitamin D deficiency 20ng/ml ELISA kit.

Statistical analysis:

IBM Corp., 2017. IBM SPSS Statistics for Windows, Version 23.0 IBM Corp., Armonk, NY) was used for data management and analysis. Numerical data were presented as mean and standard deviation or as median and range after testing the normality of the distribution. Correlation analysis tests the association of numerical variables. The chi-square and Fisher exact tests were used to compare independent categorical variables. The odds ratio, with a 95% confidence interval, measured the association between breast cancer risk and vitamin D deficiency exposure. When continuous data were distributed, Student's t-test was used for comparisons of two groups, and the Mann-Whitney test was used when data were not normally distributed. To have adjusted estimates of association, logistic regression analysis was done using the forward likelihood ratio entry and removal method for the stepwise entry and removal of covariates from the model. The odds ratio was adjusted for age, BMI, sun exposure, family income, education status, and family history of breast cancer. Variable selection was based on factors anticipated to be confounders for vitamin D's effect on breast cancer risk. Significant variables in the univariate analysis such as age at first full-term were not included as it would omit a considerable proportion from the analysis (16/140; 11.4%). All tests were two-tailed, and a p-value of 0.05 is considered significant.

Results:

Demographic data and clinical characteristics

The mean breast cancer age was 51.54 ±8.02 and 50.11 ± 5.44 in the control group (p = 0.220). There was no significant difference in family size between breast cancer cases and controls (p = 0.729).

Most breast cancer patients lived in rural locations (72.9% vs. 77.1% in the control group, p = 0.558). Most women were housewives; 87.1% of breast cancer cases and controls, were housewives, with no statistically significant difference (p = 1.00). 68.6% and 61.4% of breast cancer cases and controls were illiterate, 2.9% and 1.4% could read and write, and 22.9% and 22.9% had a high school education, with no statistical significance (p = 0.365). 85.7% of breast cancer cases and 90% of controls were married, with no significant difference (p = 0.473). 10% of breast cancer cases and 17.1% of controls had consanguinity. In 61.4% and 50% of breast cancer cases and controls, family income/month was less than 1000 EGP LE, 22.9% and 44.3% were between 1000 and 2000 EGP LE, and 15.7% and 5.7% either denied knowing or declined to answer (p = 0.012).

Most breast cancer patients and controls (62.9% vs. 72.9%) were passive smokers, and 37.1% versus 27.1% were non-smokers (p = 0.205). Regarding clinical data, the majority of women were not hypertensive: 82.9% of breast cancer cases and 78.6% of controls (p = 0.520). Also, 90% of breast cancer cases and 92.9% of the control group were not diabetics (p=0.546). Most diabetic patients and diabetic controls were treated with oral antidiabetics. Family history of breast cancer was recorded in 11.4% of cases and not recorded in the control group, with a highly significant difference (p = 0.004). A family history of cancer was found only in 8.6% of breast cancer cases and not in controls (p=0.012) Table (1).

Contraceptive, menstrual, and obstetric history:

35% of breast cancer cases and controls have taken contraceptives (p = 0.591). Most had used an IUD or oral contraception, with no significant difference (p = 0.386). Intermittent contraception was used by 64% and 81.8% of breast cancer cases and controls, respectively, p = 0. 207.Most have stopped using contraception. All breast cancer cases and controls have stopped menstruating (p = 0.601). Both groups of women do not take hormone replacements. The median age for cumulative hormonal contraceptive use was 7.5 years in breast cancer cases and eight years in controls (p = 0.227). The median age of hormonal contraception initiation was 33 years in breast cancer cases and 30 years in controls (p = 0.165) Table 2.

The median age of stopping hormonal contraception was 47 years in the breast cancer group and 48 years in the control group (p = 0.06). According to menstrual history and parity, the median age for the first full-term pregnancy was 20 in breast cancer patients and 17–30 in the control group (p = 0.015). The median number of full-term births in breast cancer cases and controls was 5 (p = 0.827). Both groups' median breastfeeding duration was eight years (p = 0.935). Menopause age was 49.0 years in breast cancer cases and 48.0 years in controls (p = 0.142).

Dietary history and sun exposure:

The majority of women were exposed daily to beneficial sunrays, 45.7% for breast cancer cases and 42.9% for the control group, with 18.6% and 28.6% weekly, 2.9% and 2.9% monthly, and 20% and 21.4% rarely, with no significant difference between the two groups (p=0.333). The median exposure to beneficial sunrays was 450 (10-3600) minutes/month (15/day) in breast cancer cases and 300 (10-1800) minutes/month (10/day) in the control group (p = 0.083). 95.7% of breast cancer cases and 98.6% of controls did not take supplements (p=0.310). One woman in the control

group gave B12 and multivitamins to only three women table (3).

Meat intake (150 g) was consumed two times per month in breast cancer cases versus one time per month in the control group without a significant difference (p = 0.118). Chicken intake (150 g) was four times per month for both study groups without a significant difference (p = 0.870). The median fish intake (150 g) was 0.08 g per month for both study groups (p = 0.094). Egg intake (as one egg) was 4/month in both research groups, with a significant difference (p = 0.038) between patients and controls. In breast cancer cases, the median cheese intake was 4.5 times per month, compared to 4 times per month in the control group (p = 0.345). Both groups drank milk once a month, with no significant difference (p = 0.449) table 3.

Comparing vitamin D serum level, BMI, and waist/hip ratio:

The median serum level of 25-hydroxy vitamin D and the median serum vitamin D level of breast cancer cases were 4.85 ng/ml vs. 4.95 ng/ml for the control group without a significant difference (p = 0.466). Because only four controls (5.7%) and one case (1.4%) had serum vitamin D levels of 30 ng/ml, we classified women as deficient if their serum vitamin D level was less than 20 ng/ml. Results showed that serum vitamin D levels were significantly lower in cases than in controls. 94.3% of cases and 78.6% of controls had vitamin D levels of less than 20 ng/ml. Only 5.7% of cases and 21.4% of controls had vitamin D levels of 20 ng/ml. The unadjusted odds ratio (OR) showed a statistically significantly increased risk of breast cancer with low vitamin D concentration (OR 4.5, 95% CI) Figures (1) and (2).

The BMI for breast cancer cases was 29.54 kg/m2 versus 29.58 kg/m2 for the control group without a significant difference (p = 0.833). Descriptive data for weight and height are shown in the same table. The median waist/hip ratio was the same (0.90 for both study groups) without a statistically significant difference (p = 0.801).

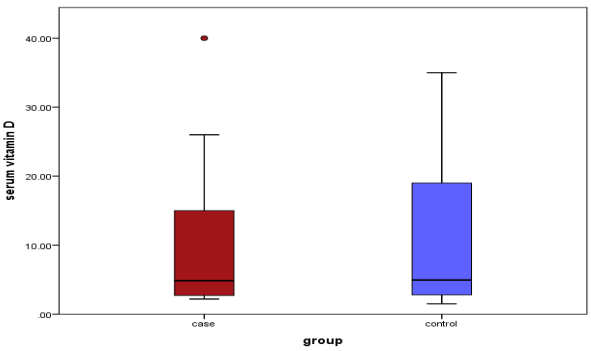

Figure 1: Box-and-whisker plot showing serum vitamin D distribution between the study groups

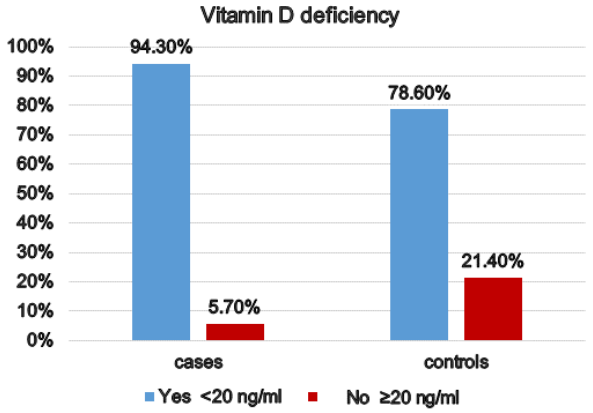

Figure 2: Bar chart showing vitamin D deficiency in the two study groups

Association between vitamin D deficiency and breast cancer risk

A logistic analysis, such as the adjusted logistic regression analysis, was done to determine if vitamin D deficiency is an independent risk factor in breast cancer. The odds ratio was adjusted for age, BMI, sun exposure, family income, education status, and family history of breast cancer. Variable selection was based upon factors anticipated to confound vitamin D's effect on breast cancer risk. Significant variables in univariate analysis, such as age at first full-term, were not included as it would omit a considerable proportion from the analysis (16/140; 11.4%). The adjusted odds ratio for breast cancer was more than five times higher for women with vitamin D concentrations of 20 ng/mL). Women with a family income of 1000–2000 LE/month have 86% less risk than those with a monthly income of 1000 LE, and those with a positive history of breast cancer are more than 14 times more at risk of breast cancer (Table 4).

Pathologic characteristics of breast cancer cases and their relation to vitamin D:

pathological characteristics of breast cancer cases Invasive ductal carcinoma (IDC) was the most common pathology (85.7%), followed by invasive lobular carcinoma (7.1%), invasive mammary carcinoma (7.1%), invasive breast carcinoma (5.7%), and mucinous mammary carcinoma (1.4%). When comparing the left and right sides of the tumor in breast cancer cases, the left side was represented by 52.9% and 47.1%. Regarding tumor stage, and according to SEER staging, the tumor was localized to the breast in 31.4% of patients, 20% showed regional spread, 7.1% showed locally advanced, and no available data accounted for 41.4% of breast cancer patients. For tumor grade, grade II showed the highest frequency of 95.7%, and grade III was 4.3%. The majority of breast cancer cases were subjected to modified radical mastectomy (75.7%), conservative surgery (1.4%), and (22.9%) biopsy, but no definitive diagnosis of breast cancer was made.

In terms of the relationship between serum 25-hydroxyvitamin D levels and clinicopathological

features of breast cancer, the median serum vitamin D level in cases with IDC was 4.85 ng/ml with a range of 2.20-40.00 and 4.80 ng/ml with a range of 2.50-26.00) in other pathologic types, with no significant difference (p=0.574). The serum level of 25-hydroxyvitamin D was not significantly different according to the SEER stage of breast cancer (p = 0.753) or tumor grade (p = 0.685).

Relationships between serum vitamin D levels and demographic, contraceptive, menstrual history, clinical characteristics, anthropometric measurements, and sun exposure history

Table 5 shows the correlation between serum vitamin D and some characteristics of breast cancer cases. An association analysis was done to assess the relationship between vitamin D and education level, family size, age, income, cumulative dose of contraception in years, age of stopped contraceptive intake in years, age of menopause, number of completed deliveries, number of years of breast feeding, and BMI. A reasonable positive correlation between serum vitamin D and waist/hip ratio was found, and it was statistically significant (r = 0.360, p = 0.002); no other significant correlation was found. Furthermore, the control group assessed the same relations between vitamin D and education level, family size, age, income, age since last contraceptive use, waist/hip ratio, and BMI. A positive correlation was found between serum vitamin D level and both educational level (fair) and cumulative dose of contraception in years (moderate). A negative correlation between serum vitamin D and age (weak), number of completed deliveries (fair), and number of years of breastfeeding (fair) was also found (table 5).

Table 1: Demographic and clinical characteristics

Variables		Group				p-value
		Cases (n=70)		Control (n=70)		
		Count	%	Count	%	
Demographic characteristics						
Age (year)	Mean ±SD	51.54±8.02		50.11±5.44		0.220
Family size	Median (range)	6.0 (1 -14)		6.0 (3 – 10)		0.729
Residence	urban	19	27.1	16	22.9	0.558
	rural	51	72.9	54	77.1	
Occupation	housewife	61	87.1	61	87.1	1.000
	employee	9	12.9	9	12.9	
Education level	Don't read/write	48	68.6	43	61.4	0.365
	Read and write	2	2.9	1	1.4	
	school education	16	22.9	16	22.9	
	college (university)	4	5.7	10	14.3	
Marital status	single	4	5.7	4	5.7	0.473
	married	60	85.7	63	90.0	
	widow	6	8.6	2	2.9	
	divorced	0	0.0	1	1.4	
Consanguinity Status	yes	7	10.0	12	17.1	0.217
Income in LE *	<1000	43 a	61.4	35 a	50.0	**0.012***
	1000-<2000	16 a	22.9	31 b	44.3	
	>2000	0	0.0	0	0.0	
	Don't know/refused to answer	11 a	15.7	4 a	5.7	
Current smoker	passive smoker	44	62.9	51	72.9	0.205
Clinical characteristics						
Hypertension		12	17.1	15	21.4	0.520
Diabetes mellitus		7	10.0	5	7.1	0.546
Treatment of diabetes	oral treatment	5	83.3	2	66.7	0.682
	insulin injection	0	0.0	1	33.3	
	oral +injection	1	16.7	0	0.0	
Family history of breast cancer		8	11.4	0	0.0	0.004*
Family history of cancer		6	8.6	0	0.0	0.012*

* p value is significant at 0.05 level,

Table 2: Contraceptive, menstrual and obstetric history of the study participants at South Egypt Cancer Institute, Assiut University, 2020

Variables	Group				P value
	Cases (n=70)		Control (n=70)		
	Count	%	Count	%	
Contraceptive history					
Contraceptive use	25.0	35.7%	22.0	34.1%	0.591
Type of contraception					
Injection	3	12.0%	1	4.5%	
IUD	10	40.0%	9	40.9%	0.386
Oral	5	20.0%	7	31.8%	
Oral+ injection	5	20.0%	1	4.5%	
Implants	2	8.0%	4	18.2%	
Regularity of contraception					0.207
Continuous	9	36.0%	4	18.2%	
Intermittent	16	64.0%	18	81.8%	
Did she stop using contraception?	17	68.0%	13	59.1%	0.558
Has menstrual cycle stopped	45	64.3%	42	60.0%	0.601
Intake of replacement hormones	70	100.0%	70	100.0%	–
No					
Cumulative intake of hormonal contraceptives in years, median (range)	7.5	(4 – 13)	8.0	(4 – 15)	0.227
Age started contraceptives in years median (range)	33.0	(24 -40)	30.0	(24 -40)	0.165
Age stopped contraceptives in years median (range)	47	(38-55)	48	(44-48)	0.605
Menstrual history and parity					
Age at menarche (years)	12.0	(10-17)	12.0	(11-17)	0.089
Age at first full-term pregnancy	20.0	(17–30)##	21.0	(17-25)#	**0.015***
Number of full-term deliveries	5.0	(0–9)##	4.0	(0 – 8)#	0.827
Number of years of breast feeding	8.0	(0–18)##	8.0	(0–16)#	0.935
Age at menopause (years)	49.0	(40-56)	48.0	(46-58)	0.142

*p value is significant at 0.05 levels,
Number of women involved is 63,
Number of women involved is 61

Table 3: Sunray exposure and dietary supplementation

Items	Group				p-value
	Cases (n=70)		Control (n=70)		
	Count	%	Count	%	
Exposure to beneficial sunray:					
Daily	32	45.7	30	42.9	
Weekly	13	18.6	20	28.6	
Monthly	2	2.9	2	2.9	0.333
Winter only	9	12.9	3	4.3	
Rarely	14	20.0	15	21.4	
Sunray exposure per month (minutes)					
Median (range)	450 (10-3600)		300 (10-1800)		0.083
Supplement intake:					
Yes	3	4.3	1	1.4	0.310
No	67	95.7	69	98.6	

Table 4: Binary logistic regression analysis for association of vitamin D deficiency with breast cancer risk

Variables	B	S.E.	Wald	p value	OR	95% C.I. for OR	
						Lower	Upper
Vitamin D <20 ng/ml	1.680	.631	7.099	**0.008***	5.367	1.559	18.473
History of breast cancer	2.678	1.163	5.307	**0.021***	14.56	1.491	142.15
Income <1000 LE			9.736	**0.008***			
Refused to answer	-1.010	0.660	2.341	0.126	0.364	0.100	1.328
1000 - 2000 LE	-1.958	0.697	7.883	**0.005***	0.141	0.036	0.554
Constant	-0.391	0.800	0.238	0.625	0.677		

B= regression coefficient, S.E = Standard error, OR = odds ratio, CI =confidence interval

Table 5: Correlations of serum vitamin D with demographic, contraceptive, menstrual history, clinical characteristics, anthropometric measurement, and sun exposure history

Items	Serum vitamin D level			
	Cases (n=70)		Control (n=70)	
	r- value	p- value	r- value	p- value
Educational level	0.134	0.269	**0.243**	**0.043***
Family size	-0.021	0.864	-0.104	0.390
Age (years)	-0.223	0.063	**-0.256**	**0.033***
Income in LE	0.225	0.061	-0.017	0.888
Cumulative dose of contraception (years)	-0.085	0.692	**0.507**	**0.016***
Age stopped contraceptives in years	-0.288	0.279	0.068	0.825
Age of menopause	-0.267	0.076	-0.247	0.114
Number of completed deliveries	-0.222	0.086	**-0.307***	**0.014***
Number of years of breast feeding	-0.238	0.068	**-0.373**	**0.003***
BMI	0.024	0.845	-0.014	0.906
Waist/hip ratio	0.360	**0.002***	0.152	0.208
Cumulative period of sun exposure (mins/day)	0.076	0.576	0.178	0.190

Discussion:

In the present study, our main goal was to assess the association of circulating vitamin D level with risk of breast cancer through the inclusion of 70 breast cancer cases and a 70-person matched control group at the South Egypt Cancer Institute, Assiut University.

Demographic characteristics:

Age, family size, residence, occupation, marital status, and education were similar. Khater et al. (2019) discovered that the mean age of BC patients at the NCI, Cairo University was 47.511.0 years (range: 26–80 years) [10]. Also, 45% of patients in research at Al-Azhar Assiut University were between 50 and 60 years old, and their mean age was 50.6 [11].

Gabr et al. (2017 reported that 68.5% of breast cancer cases and 65% of healthy control women had more than three children, indicating parity in both groups [12]. Harper et al. (2009) think the residential environment may affect breast cancer incidence and death through the geographic distribution of risk factors, access to quality and timely healthcare, and psychosocial pathways involving stress and social support [13].

Having a breast cancer-diagnosed mother, sister, or daughter doubles the risk. This risk increases if more close relatives have breast cancer or if a relative is under 50. In the current study, 11.4% of breast cancer patients had a positive history of the disease, which, according to multivariate analysis, increases the risk of breast cancer by more than 14 times (OR, 95% CI: 14.6, 1.5–142.2).

Dietary history and sun exposure:

In similar case-control research in Pakistan, breast cancer cases and controls had 59% and 69% daily

exposure to helpful sunrays, respectively. Though the proportion of exposed women was higher than in the present study, the overall benefit time was the same: less than 20 minutes per day [14].

In contrast to our results, Engel et al. found evidence of a link between sun exposure and breast cancer risk [15]. Wu et al. (2013) showed that sunshine exposure might be associated with a lower risk of breast cancer without clear evidence of alteration by the vitamin D receptor (VDR) variation, a major vitamin D mediator [16]. Furthermore, regular sun exposure may reduce the incidence of breast cancer [17]. Asakura et al. (2020) discovered a significant positive relationship between UV exposure period and serum 25(OH)D3 concentration [18].

In this research, supplements were not linked to breast cancer risk. In both study groups, just 4 people received supplements; none received vitamin D. A systematic analysis found no strong evidence for vitamin supplements' cancer-prevention benefit, citing few relevant studies and inconsistent results [19]. Except for occasional egg consumption monthly, where breast cancer cases ate more eggs than controls, we found no significant link between vitamin D-containing dietary items and breast cancer risk. Sofi et al. (2018) observed that occasional egg consumption was lower than twice to once a month but higher than daily to once a week [20]. Changes in food habits after a cancer diagnosis may explain the disparity between this study and others, especially in disadvantaged places like the Assiut governorate.

Protein, fat, calcium, fruits, and vegetables did not increase breast cancer risk. Sofi et al. (2018) reported similar results and indicated that due to the anti-cancerous effects of fruits and vegetables, an inverse yet non-significant correlation was discovered between daily consumption of fruits and vegetables and breast cancer risk [20].

Vitamin D:

The present investigation indicated a low median serum vitamin D level in breast cancer cases and the control group. Only four controls (5.7%) and one case (1.4%) had blood vitamin D levels of 30 ng/ml. When deficiency was defined as vitamin D 20 ng/ml, 94.3% of cases and 78.6% of controls had it, for an overall rate of 86.4%. Serum vitamin D is normally 30–100 ng/ml. Comparable results were found in two US studies to assess the prevalence and correlation of vitamin D deficiency in the US population, one of which was conducted on 4495 adult participants by Forrest and Stuhldreher (2011) [21], where vitamin D deficiency was defined as serum 25-hydroxyvitamin D concentrations of 20 ng/mL (50 nmol/L).

Vitamin D deficiency was more common in people with no college education, obesity, poor health, hypertension, low HDL cholesterol, or not drinking milk daily. In the present study, blood vitamin D level was positively connected with educational level and cumulative dose of contraceptives in years and negatively correlated with age, number of completed births, and number of years breastfeeding. The other US study evaluated African Americans' health disparities in vitamin D insufficiency. It has been linked to a higher incidence of health problems and mortality among African Americans than among European Americans. They are deficient in vitamin D by a factor of 15 to 20 [22].

A meta-analysis of 44,717 participants from five South Asian nations reported a pooled prevalence of deficiency of 68% (95% CI: 64–72%) with significant heterogeneity and an average vitamin D level of 19.15 ng/mL (weighted standard deviation of 11.59 ng/mL). Pakistan had the greatest prevalence of vitamin D deficiency (73%), followed by Bangladesh (67%; 95% CI: 50 to 83%), India (67%; 95% CI: 61 to 73%), Nepal (57%; 95% CI: 53 to 60%), and Sri Lanka (48%; 95% CI: 41 to 55%). Variability in sunshine exposure due to geography was proposed as a possible cause [23].

Females in Assiut, Egypt, have a significant incidence of vitamin D insufficiency, despite ample sunlight. This can affect food and clothing. Botros et al. (2019) found that females are the most affected by vitamin D deficiency in Egypt. Urbanization and socioeconomic reasons explain the tendency [7].

Different research has assessed vitamin D insufficiency and breast cancer risk. I of 1.56–18.47. In a 2017 Pakistani case-control study by Shaukat et al., serum vitamin D levels were low in both patients and controls. Vitamin D risk factor is a risk for breast cancer, with an adjusted hazard 1.99–30.58.8 (1.99–30.58) for women with vitami20 ng levels of 20ng/ml. [14]. Shaukat et al. (2017) found a 5.37 adjusted odds ratio (95% CI) (1.56-18.47) [14].

In Saudi Arabia, Yousef et al. (2013) discovered that breast cancer cases had lower vitamin D serum levels than controls. The adjusted AORs (95% CIs) for invasive breast cancer were 6.1 (2.4–15.1) for women with a serum 25(OH)D concentration of 10 ng/mL and 4.0 (1.6–10.4) for those with 10–20 ng/mL (p-trend = 0.0001) [24]. Atoum and Alzoughool (2017) showed an inverse connection between vitamin D and breast cancer risk [25].

Kim and Je (2014) observed that high vitamin D levels were weakly connected with decreased breast cancer risk but were highly associated with better breast cancer survival [26]. In a retrospective study of patients with HER2-negative non-metastatic breast cancer receiving adjuvant chemotherapy, Zeichner et al. (2015) found that vitamin D supplementation was more effective than the control group [27]. Ahmed et al. (2019) found that chemotherapy-treated breast cancer patients need vitamin D [28].

Over a 5-year period, O'Brien et al. (2017) discovered that high serum 25(OH)D levels and regular vitamin D supplement use were associated with a lower incidence of incident postmenopausal breast cancer. This data supports the theory that vitamin D prevents breast cancer [29]. Krishnan et al. (2010) interpreted vitamin D supplementation's role in reducing breast cancer risk as downregulating estrogen receptor expression and attenuating hormone production and signaling [30]. According to IOM guidelines, 20 ng/mL is an adequate dietary dose of vitamin D for 97% of the population. However, the normal range is for bone

health, not the vitamin's extra skeletal benefits [31]. Recent research suggests supplementing with 1000 IU/d of vitamin D to attain 35 ng/mL [32].

Relation of serum vitamin D to clinicopathological features:

The current study found no link between vitamin D and tumor clinicopathological features. Imtiaz et al. (2012) found no relationships between tumor features (histology, grade, stage, and receptor status) and vitamin D levels [8]. Kim et al. (2011) examined serum 25-OHD in 310 Korean women with breast cancer from June to December 2006 and found that those with insufficient levels had a higher probability of recurrence (P = 0.002). 25-OHD concentration was inversely linked with the prognosis of patients with luminal A (p = 0.012) and B (p = 0.023) cancer subtypes, but not with Her2/neu-enriched (p = 0.245) or triple-negative (p = 0.879) cancer subtypes [33]. This connection remained after adjusting for age, tumor size, nodal status, and estrogen receptor status (HR = 3.97; 95% CI = 1.77–9.61). Thanasitthichai et al. (2015) analyzed 200 breast cancer cases in Thailand from 2011–2012. Vitamin D levels decreased as tumor stage increased [34].

Abdulrazzaq and Ahmed (2020) studied 50 breast cancer patients in Erbil Governorate, Iraq, and found an association of breast cancer with vitamin D levels. The mean serum 25-hydroxyvitamin D level decreased with premenopausal stage (p = 0.04) and HER2/neu + immune-expression (p = 0.008), particularly in postmenopausal women (p = 0.035). The mean 25-hydroxyvitamin D level was lowest in HER2/neu-enriched subtypes (p = 0.033) [35].

Additionally, obesity and metabolic syndrome are linked to vitamin D status. Since fat cells express vitamin D receptors (VDR) and obesity is a cancer risk factor, vitamin D actions in adipocytes may contribute to their defensive cancer characteristics [36]. Matthews et al. (2016) explored the role of VDR in adipose tissue, chiefly in the setting of the mammary gland, in adipose-specific VDR deletion (CVF mice). Adipose deletion of VDR dramatically increased mammary epithelial cell density and branching, confirming that VDR in mature fat cells modulates the metabolic response to high-fat diets and exerts antiproliferative activities on mammary epithelial cells [36]. Matthews et al. (2016) found no association between BMI and vitamin D level but a favorable correlation with waist-to-hip ratio. In the current study, vitamin D level and waist/hip ratio correlated positively only in breast cancer patients [36].

A study on vitamin D, genetic and environmental variables had positive results [37]. Nelson et al. (2009) suggest that estrogen-containing oral contraceptives increase vitamin D 25-hydroxylase activity in the liver [38]. Møller et al. (2013) believed that the rise was due to an increase in circulating vitamin D binding protein (VDBP) [39]. It is unclear whether this 25(OH)D biochemical reaction occurs independently or in conjunction with vitamin D activity on bone metabolism. Bioactivity may not increase if the increase in 25(OH)D is due to an increase in circulating VDBP and bound vitamin D. We use 25-OHD to detect vitamin D insufficiency, which is problematic. Rising estrogen levels may mask a vitamin D bioactivity deficiency [39].

Conclusion:

Except for egg consumption, dietary history and supplement intake were similar between the two groups. Most breast cancer patients and control group women got daily sun exposure, but not enough. Most breast cancer patients and control women had low serum vitamin D levels, but more cases had levels less than 20 ng/ml. After adjusting for age, BMI, sun exposure, family income, education status, and breast cancer family history, serum vitamin D levels of 20 ng/ml increased breast cancer risk more than fivefold.

In breast cancer cases, serum vitamin D levels were unrelated to pathologic features but positively correlated with the waist/hip ratio. In the control group, serum vitamin D level was positively correlated with education and cumulative contraceptive dose but negatively correlated with age, the number of births, and breastfeeding.

Study strengths and limitations:

The study strengths included:

This study is unique in that it focuses on Upper Egypt females, a group at higher risk of vitamin D deficiency and with low 25(OH)D concentrations.

The short window for bio-sampling that reduced seasonal effects was an additional strength.

The availability of demographic and lifestyle data during a face-to-face visit with each woman

The opportunity to have measured BMI and waist/hip ratio as covariates in the analysis was an additional strength of our methods.

The collection of detailed dietary intakes would also be beneficial. However, there is sparse evidence that dietary vitamin D alone significantly modifies circulating concentrations in depleted individuals.

The study limitations included:

The absence of bio samples prior to diagnosis, as well as the use of a single 25(OH)D measurement to determine status.

Multiple measures of 25(OH)D would be preferable for determining long-term average 25(OH)D status.

Participants' illiteracy, self-reporting of dietary history, and refusal to disclose income could all result in measurement bias.

Sample size could be another limiting factor.

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