ORIGINAL ARTICLE

VITAMIN-D STATUS AT BREAST CANCER DIAGNOSIS: CORRELATION WITH SOCIAL AND ENVIRONMENTAL FACTORS AND DIETRY INTAKE

Saba Imtiaz, Neelam Siddiqui

Department of Medical Oncology, Shaukat Khanum Memorial Cancer Hospital and Research Centre, Lahore, Pakistan

Background: Serum levels of vitamin-D are low in breast cancer patients. Vitamin-D levels have inverse correlations with postmenopausal state, obesity, poor sun exposure and low intake of vitamin-D rich diet. The objective of this study was to quantify levels of vitamin-D in serum to determine the degree of vitamin-D deficiency in breast cancer patients compared with age matched controls and to observe the association between serum 25-OH vitamin-D levels and personal and social parameters, BMI, amount of sun exposure and dietary intake. **Methods**: In this cross-sectional analytical study all newly diagnosed breast cancer female patients were recruited into the study over a period of 6 months. Age matched healthy females were also recruited as a control group. Personal and social data was documented on to a *pro forma*. Sun exposure was determined by mid-day exposure to direct sunlight. Serum 25-OHD levels were studied by ELISA technique on the blood samples. The dietary information was collected by recall over the last 1 year. **Results**: Vitamin-D deficiency was found in 99% breast cancer females and 90% in healthy females. Mean serum vitamin-D level was 9.6±5 ng/ml and 15.2±10 ng/ml for cases and control group respectively. All breast cancer and 95% healthy females with BMI >30 were found to be vitamin-D deficient. Menopausal state, parity, parda (veil) observation, area of living and sun exposure did not affect vitamin-D status in either group. Egg, fish and cheese intake revealed correlation with vitamin-D deficiency. Forty percent healthy females were found to have deficient serum vitamin-D levels despite being on supplement. **Conclusion**: Vitamin-D deficiency was highly prevalent among breast cancer females. Serum 25 OHD levels exhibited an inverse correlation with high body mass index and vitamin-D rich diet.

Keywords: Vitamin-D deficiency, breast cancer, 25-OHD, sun exposure, body mass index

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INTRODUCTION

Breast cancer is the most commonly diagnosed cancer among women and it is also a leading cause of death among women globally.[1] According to a study, in Pakistan one in five of every female diagnosed with cancer is a breast cancer patient.[2] Due to the magnitude of this disease, considerable research effort is directed towards identifying preventable breast cancer risk factors.

Vitamin-D, known to regulate calcium and bone homeostasis has shown diverse biological effects relevant to immune system and carcinogenesis.[3] Vitamin-D exerts its anti-proliferative effect by binding to vitamin-D receptors (VDR) which are found in most tissues and cells of the body. Vitamin-D deficiency is being associated with increased risk of several epithelial cancers including breast, stomach, colon and Prostate.[4]

Modest amounts of vitamin-D come from dietary sources and 90% of it is produced when ultra violet B (UVB) light from the sun hits a precursor molecule in the skin. Vitamin-D from either source undergoes a series of hydroxylation steps first in the liver to yield 25-hydroxyvitamin-D (25-OHD), the major circulating metabolite and later in the kidney to produce 1, 25-dihydroxyvitamin-D [1, 25- (OH) 2D], the most biologically active form and natural ligand for VDR. Extra renal vitamin-D activation occurs in other target tissues like colon, prostate and breast which express the activating enzyme (1α-hydroxylase) and the VDRs, to locally regulate cell turnover.[3] Numerous preclinical studies have shown that vitamin-D inhibits cell proliferation, induces differentiation and apoptosis, and has antiangiogenesis effects in normal as well as malignant breast cells.[4] Measurement of the circulating concentration of 25-OHD provides an integrated measure of vitamin-D from all sources. It has a half-life of 2 months and is considered to be the best indicator of vitamin-D body stores.[3–6]

Research suggests that women with low serum levels of vitamin-D have a higher risk of breast cancer.[7,8] Women with serum levels of 25-OHD higher than 50ng/ml had a 50% lower risk of breast cancer compared to those with serum values less than 30ng/ml.[8] Other studies report that high serum vitamin-D levels at early breast cancer diagnosis correlate with lower tumour size and better OS, and improved breast cancer-specific outcomes, especially in postmenopausal patients.[9,10] The breast cells have

VDRs in their nuclei and it is postulated that polymorphism of genes for these VDRs may result in increased risk for breast cancer.[11]

There are various factors that impact the cutaneous vitamin-D synthesis. The main determinant is the angle of the sun. The more directly overhead the position of the sun the more UVB is available for vitamin-D synthesis. Latitude, season, time of day, atmospheric pollution and skin colour all affect the availability of UVB. Closer to the equator, summer season and mid-day, more UVB is available.[12] Hence, less time is needed in the sun to make vitamin-D. However, there is no recommended duration of sun exposure for optimal vitamin-D synthesis but there are suggestions that approximately 10–30 minutes of direct sun exposure between 10 AM to 3 PM at least twice a week will lead to sufficient vitamin-D synthesis.[12,13] Ecological studies have associated increased solar UVB irradiation with lower breast cancer incidence and mortality.[7]

It has also been shown in various studies that obese people with a body mass index (BMI) >30 generally have low serum levels of vitamin-D.[14,15] This high prevalence of vitamin-D deficiency is primarily due to sequestration of circulating vitamin-D into the high quantity of subcutaneous tissue in obese individuals.[15]

Food sources that naturally contain appreciable amount of vitamin-D include milk, yogurt, butter, cheese, fish and eggs. Vitamin-D supplements in daily recommended dosages (200–600 IU) are widely and inexpensively available over the counter. In one study daily vitamin-D intake of >800 IU was found to be associated with reduced breast cancer risk particularly in postmenopausal women.[16] An average of 2200–3000 IU of vitamin-D per day from sun exposure, food or supplements is needed to maintain adequate systemic levels of >30 ng/ml for beneficial effects on health.[17]

There is dearth of published data on prevalence of vitamin-D deficiency in breast cancer from South East Asia; hence giving us a rationale to conduct this study. Our main objective was to quantify levels of vitamin-D in serum to determine the degree of vitamin-D deficiency in breast cancer patients compared with age matched controls. The other aim of our study was to observe the association between serum 25-OH vitamin-D levels and personal and social parameters, BMI, amount of sun exposure and dietry intake.

MATERIAL AND METHODS

This was a cross sectional analytical study. All newly diagnosed and histopathologically confirmed breast cancer female patients who presented to Medical Oncology department at Shaukat Khanum Cancer Hospital Lahore were recruited into the study as “Cases” over a period of 6 months (March 2011–August 2011). Age matched healthy females who accompanied the non-breast cancer patients to hospital were recruited as the “Control group”. Women receiving or who had received vitamin-D replacement therapy in therapeutic doses (20,000 IU/2wkly) in the last 1 year were excluded from the study. Personal and social data was documented by direct questioning on to the *pro forma* for the study population. Women were considered premenopausal uptil age 45 years and those who were cycling regularly. Postmenopausal women were those who had experienced their last menstrual cycle at least one year before. Urban living was considered living in cities and towns while rural dwellers were those living in villages. Sun exposure was determined by mid-day (10 am till 3pm) exposure to direct sunlight in hours. It was either none, rare (<1 hour per month), occasional (<1 hour per week) and frequent (>1 hour per week). Vitamin-D (25-$(OH)_2$ D) levels were studied by ELISA technique on the blood samples drawn of the study population at their initial presentation and the values were documented in ng/ml. Vitamin-D deficiency was considered at serum level less than 30ng/ml and optimal levels were more than 30 ng/ml. Body mass index (BMI) was calculated as a person's weight in kilograms divided by her height in meters squared. Normal weight is considered at a BMI of 18.5–24.9, underweight is <18.5, overweight is 25–29.9 and obese at BMI of >30. The dietary information was collected by recall over last 1 year. A common unit /portion size was specified for each food item. Intake of vitamin-D rich foods like milk, eggs and yogurt was documented as regular (daily) or irregular (once per week or less often). Cheese and fish were categorized as none, rare (once in 6 months) or occasional (once per month). Supplemental vitamin-D (200–400 IU) intake was documented as none, daily or monthly. The study was approved by the hospital ethics review board and informed consent was obtained from all the study participants. Data analysis was done by using SPSS-19. The demographic variables and the descriptive measures of cases and controls were presented in frequency and percentages. The association between vitamin-D deficiency with personal and environmental factors was determined by using Chi-square test. The relation of vitamin-D status with sun exposure, dietary intake and supplements amongst the cases and controls was calculated by using chi-square. P-value of ≤ 0.05 was considered statistically significant, between 0.05 and 0.10 was considered non ignorable and >0.10 were considered definitely insignificant.

RESULTS

A total of 200 women were enrolled in the study with 100 in each group. The mean age of Cases was 47.6±9.6 years and of Control group was 46±12 years. Mean serum vitamin-D level was 9.6±5 ng/ml and 15.2±10 ng/ml for Cases and Control group respectively. Most of the women were married, multiparous and urban dwellers in either study group.

Vitamin-D deficiency (<30ng/ml) was found in 99 breast cancer females (Cases) while only one female had optimal levels. In the Control group 90 women were found to be deficient and 10 had optimal serum vitamin-D levels. The p-value was 0.003. (Table-1)

Sixty six (100%) multiparous breast cancer females were vitamin-D deficient. 46 premenopausal and 53 postmenopausal women were deficient and only one postmenopausal case had optimal serum vitamin-D levels. Seventy two (98%) urban and 27 (100%) rural dwellers had deficient vitamin-D levels. Forty two (98%) out of 43 parda (veil) observing while 57 (100%) non-parda observing females were found to be vitamin-D deficient. By occupation 90 breast cancer patients were housewives and out of them 89 (99 %) were deficient for vitamin-D. Thirty (100%) breast cancer females with BMI >30 had deficient serum vitamin-D levels. 24 (96%) out of 25 breast cancer females with normal BMI were vitamin-D deficient. Eighteen (95%) women with frequent sun exposure and 81 (100%) females with occasional or rare exposure were deficient.

In the control group, amongst the 47 multiparous women 42 (89 %) were found to have deficient serum vitamin-D levels. Equal number (n=45) of pre and postmenopausal females had vitamin-D deficiency. Ninety two percent urban dwellers and 81% women living in rural areas were found to be deficient in vitamin-D. Eighteen (95%) out of 19 healthy females with BMI >30 were found to be vitamin-D deficient and 18 (67%) out of 27 females with normal BMI had deficient vitamin-D levels with *p*-value <0.001. Regarding sun exposure, 96% females with no exposure and 88% females with frequent exposure to sunlight revealed deficient serum vitamin-D levels with *p*-value 0.77. (Table 2)

Regarding dietry intake, 34 (97%) out of 35 breast cancer females who took daily milk were found to be vitamin-D deficient. Twelve (92%) out of 13 breast cancer females with regular yogurt intake had deficient vitamin-D levels. Twenty two (96%) out of 23 women eating egg daily were found to have vitamin-D deficiency. Ninety nine percent women did not consume fish or cheese and were vitamin-D deficient. One out of 90 cases taking no vitamin-D supplement showed optimal serum vitamin-D level and 10 (100%) cases irrespective of vitamin supplement were found to be deficient. No test of significance was applied to these findings.

In the healthy female group, milk and yogurt intake did not show statistically significant correlation with serum vitamin-D levels. Daily egg was taken by 40 females with 32 (80%) vitamin-D deficient. *p*-value was 0.006. Occasional intake of cheese and fish resulted in 67% and 100% optimal serum vitamin-D levels with *p*-value 0.04 and <0.001 respectively. Nine (65%) out of 11 healthy females with a monthly vitamin-D supplement were found to be deficient in serum levels while 2 (40%) out of 5 females with a daily vitamin supplement were deficient. *p*-value was <0.001. (Table-3)

Table-1: Frequency of vitamin-D status

Serum vitamin-D level	Breast Cancer Females (Case)		Healthy Females (Control)	
	No.	%	No.	%
Deficient	99	99.0%	90	90.0%
Optimal	1	1.0%	10	10.0%
***p*-value**	0.003			

Table-2: Association of Vitamin-D status with personal and social parameters in the control group

	Serum vitamin-D level				
	Deficient		Optimal		
	No.	%	No.	%	
Parity					
None	15	88.2	2	11.8	0.908
Upto 3	33	91.7	3	8.3	
>3	42	89.4	5	10.6	
Menopausal state					
Premenopausal	45	93.8	3	6.3	0.223
Postmenopausal	45	86.5	7	13.5	
Area of living					
Rural	13	81.3	3	18.8	0.239
Urban	77	91.7	7	8.3	
Social status					
Observes Parda	31	91.2	3	8.8	0.776
Do not observe Parda	59	89.4	7	10.6	
Occupation					
Housewife	57	89.1	7	10.9	0.838
Office work	32	91.4	3	8.6	
Field work	1	100.0	0	.0	
BMI					
<18.5	5	100.0	0	.0	<0.001
18.5–24.9	18	66.7	9	33.3	
25.0–29.9	49	100.0	0	.0	
>=30	18	94.7	1	5.3	
Sun Exposure					
None	21	95.5	1	4.5	0.775
Rare	24	88.9	3	11.1	
Occasional	31	88.6	4	11.4	
Frequent	14	87.5	2	12.5	

Table-3: Association of Vitamin-D status with dietary intake and Vitamin-D supplement in the Control group

	Serum vitamin-D level				
	Deficient		Optimal		
	No.	%	No.	%	
Milk					
Irregular	50	94.3	3	5.7	0.121
Regular	40	85.1	7	14.9	
Yogurt					
Irregular	74	92.5	6	7.5	0.123
Regular	16	80.0	4	20.0	
Cheese					
None	83	92.2	7	7.8	0.044
Rare	6	85.7	1	14.3	
Occasionally	1	33.3	2	66.7	
Egg					
Irregular	58	96.7	2	3.3	0.006
Regular	32	80.0	8	20.0	
Fish					
None	84	96.6	3	3.4	<0.001
Rare	6	54.5	5	45.5	
Occasionally	0	0.0	2	100.0	
Vitamin-D Supplement					
None	79	97.5	2	2.5	<0.001
Daily	2	40.0	3	60.0	
Monthly	9	64.3	5	35.7	

DISCUSSION

Our results for vitamin-D deficiency in the healthy control group lie within the range reported in Pakistani literature with a reported prevalence of 85–98% as observed by numerous studies.[5,6] The association between breast cancer risk and serum levels of vitamin-D has not been looked at in Pakistan and our study is one of the few works being reported so far. In our study the mean serum vitamin-D levels of 9.6 ng/ml and 15.2 ng/ml are observed in breast cancer and healthy females respectively. There have been lower mean 25OHD levels in breast cancer population compared to healthy controls and the risk association has been stronger for breast cancer with lower levels.[7,8] We have found statistically significant deficiency in vitamin-D status in our two study groups.

Due to the observational nature of our research and the finding that 99% of breast cancer females (Cases) were deficient in vitamin-D, we did not estimate correlation with personal, social and dietary factors but we feel it important to analyse and discuss the said associations in the healthy (Control) group of the study population.

Multiparity partially explains that women would be deficient in Vitamin-D due to suboptimal nutrition after undergoing multiple parturitions but the deficiency was not of statistical significance when compared with nulliparous or women with less off-spring. There was a marginal increase in premenopausal women as being vitamin-D deficient which is contrary to findings in other studies and the reasoning remains elusive here. Research has shown that low levels of serum 25OHD are the norm rather than the exception in South East Asia and its prevalence is more common in the urban population.[5,12] It is generally understood that there is less atmospheric pollution in rural areas, more intense UVB light is available for cutaneous vitamin-D synthesis and hence rural dwellers if adequately exposed to sunlight should have optimal vitamin-D levels. However, in our study vitamin-D deficiency was seen in people coming from villages and cities alike. In our part of the world women are fully covered with clothes following the social and religious norms with only hands, feet and face exposed, hence providing for reduced cutaneous synthesis of vitamin-D. Although more women in the study did not observe parda, i.e., they had their hands, feet and face exposed; vitamin-D deficiency did not differ in either subgroup. More women were housewives, confined to their homes and did not have adequate sunlight exposure but their association with vitamin-D deficiency was not found to be significant compared to the office goers or field workers. More than 50% women had BMI above the expected normal and all of them were vitamin-D deficient, revealing a statistically significant causal link of low vitamin-D levels with obesity. Lower physical activity and obesity is considered an important risk factor for vitamin-D deficiency.[14,17] Obesity is a known contributor of metabolic syndrome with vitamin-D deficiency also proving its role in its pathogenesis.[15] Higher BMI is also known to be associated with an increased risk of breast cancer and a more advanced stage of the disease. Treatment as well as overall and disease-free survival for breast cancer is adversely affected by the presence of obesity.[18]

Women with mid-day sun exposure for more than one hour per week showed similar degree of vitamin-D deficiency as those with rare and occasional exposure. We could not find a significant association between the amount of sun exposure and vitamin-D status. This is in contrast to data showing solar effect on 25OHD levels. Although we interviewed our study population in the summer season (March–August) with maximum sun light, the clothing preference, dark skin of our population, airborne pollutants, narrow city streets and reduced outdoor activity impacted on the decreased UVB exposure and hence low vitamin-D levels.

Women with regular daily intake of milk and yogurt compared to those who were not regular revealed vitamin-D deficiency alike. Egg, cheese and fish although taken by fewer women did show significant association with vitamin-D status. Similarly, regular oral vitamin-D supplementation also showed a positive association with serum vitamin-D levels. Correlative studies of vitamin-D intake via food and supplements are inconsistent but generally indicate a reduced risk of breast cancer particularly in premenopausal females.[16,19]

Our study has its strengths and limitations. The strengths include its prospective nature with a relatively comprehensive detail of personal and social characteristics and dietary information. Serum 25OHD levels were measured independently in the hospital pathology laboratory where the pathologists were blinded to the clinical data. The principal limitation of our study was its cross sectional design .The one female that had optimal levels of vitamin-D in the breast cancer group was a married postmenopausal housewife living in the city. She had a normal BMI, reported frequent sun exposure and had a regular daily intake of milk, yogurt and egg. With only one out of 100 cases with optimal vitamin-D status the causative nature of the associations cannot be established. Relatively small size of our study, limited our ability to detect statistically significant results. The sun exposure index should have been calculated according to a standard formula using fraction of body surface area exposed to sunlight.

CONCLUSION

Vitamin-D deficiency is highly prevalent among breast cancer females. Serum 25 OHD levels exhibited an indication of inverse correlation with body mass index, vitamin-D rich diet and supplements. Few epidemiological efforts have been made to investigate the association between Vitamin-D concentration and breast cancer risk in Asian women. Well-designed larger clinical trials are needed to look at the social and dietary associations in breast cancer women with vitamin-D.

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Address for correspondence:

Dr. Saba Imtiaz, 7-A, Block R-3, M.A Johar Town, Lahore, Pakistan. **Cell**: +92-300- 4235944
Email: sabaimtiaz@hotmail.com