RESEARCH ARTICLE

A case control study to determine the frequency of vitamin d deficiency in carcinoma breast in females of local population of Rawalpindi and Islamabad area

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Abstract

Objective: To determine the frequency of vitamin D deficiency in breast carcinoma cases.

Method: The case-control study was conducted at the Department of Pathology, Pak-Emirates Military Hospital, Rawalpindi, between February and December 2024, and comprised females aged 20-75 years newly diagnosed with breast carcinoma in group A and age-matched healthy controls in group B. Sociodemographic data and clinical history were obtained, and serum levels of 25-hydroxyvitamin D were tested using electrochemiluminescense immunoassay. Deficiency in vitamin D was considered serum level <25nmol/L. Data was analysed using SPSS 21.

Results: Of the142female subjects, 71(50%) were in group A with mean age 46.9±16.3 years, and 71(50%) were in group B with mean age 42.4±15.9 years (p=0.797). Vitamin D levels were also lower in group A patients compared to those in group B (p<0.001). Vitamin D deficiency was observed in 33(46.5%) cases compared to 5(7%) controls, with supplement use being less among the cases 11(15.5%) compared to the controls 28(39.4% (p=0.001). There was a high level of correlation between vitamin D deficiency and positive family history of breast carcinoma (p<0.001).

Conclusion: Deficiency of serum 25-hydroxyvitamin D contributed to the higher risk of breast carcinoma, while vitamin D supplements had preventive benefits.

Keywords: Vitamin D deficiency, Serum vitamin 25 hydroxyvitamin D, Breast carcinoma. (JPMA 76: 1283; 2026)

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Introduction

Vitamin D is a vital nutrient that serves as a precursor to a potent steroid hormone that has an important role in bone metabolism. Recent advances have elucidated significant vitamin D involvement in the regulation of disease processes, particularly in the onset and development of various chronic medical conditions, including cancer.[1] Its synthesis in body is greatly aided by sunlight, and it acts as a pleiotropic hormone. It has been found that almost all cell types have vitamin D receptors (VDR), and all vital organs and tissues of the body benefit from vitamin D.[2] Dendritic cells and antigen-presenting cells (APCs), involved in the differentiation of T lymphocytes, confirm the influence of vitamin D on cell differentiation.[3]

One of the nuclear receptors found in the breast tissue, the VDR is triggered by its hormone ligand, 1, 25-dihydroxyvitamin D (1,25D). The VDR gene has been shown to include a number of single nucleotide polymorphisms (SNPs), and some of them are associated with the development of tumours.[4] The concentration of serum 25-hydroxyvitamin D (25[OH]D) is the most precise marker of vitamin D levels in humans. Clinical outcomes are influenced by the sensitivity of VDR, duration of vitamin D deficiency, individual calcium needs, and dietary calcium intake. A significant frequency of vitamin D insufficiency has been reported by multiple studies in Pakistan.[5] Worldwide, vitamin D insufficiency prevalence is estimated to exceed a thousand million individuals, and it is more prevalent in developing nations. It is particularly classified as one of the five most prevalent paediatric health issues.[6]

Cancer is a critical condition marked by uncontrolled cellular proliferation, with rising mortality and diagnostic rates. It is estimated that by 2040, there will likely be around 28.4 million freshly diagnosed cases of cancer globally. In 2020, there were around 10 million cancer-related deaths worldwide and 19.3 million new cancer diagnoses (excluding non-melanoma skin cancer). The number of breast carcinoma (BC) cases has risen to 2.26 million cases worldwide.[7] Approximately 42,000 deaths were also reported due to BC in the same year, and over 268,000 new cases were registered. Globally, women make up 49.5% of the population, with the majority of these women being aged >60 years. One study indicated that about 2.1 million females were newly diagnosed with BC, equating to one new case every 18 seconds. Additionally, 626,679

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individuals died from this malignancy.[8] Vitamin D supplementation and serum 25 hydroxy vitamin D2(25[OH]2D) concentrations have been found to be extremely reliable independent indicators of the risk of BC. Several studies conducted in developing nations have demonstrated that females with serum vitamin D levels <30ng/ml are 50% more likely to develop BC, and those with levels >50ng/mL are 50% less likely to do so. Serum levels of vitamin D and oral calcium intake have been shown to lower BC incidence in premenopausal females, but these effects were not statistically significant. Research indicates that women with locally advanced BC exhibit acute serum 25(OH)2D deficiency compared to those with early cancer stages.[9]

Despite several nutritional and food safety initiatives, vitamin D deficiency has a high prevalence in the twin cities of Rawalpindi and Islamabad in Pakistan.[10] The current study was planned to determine the frequency of vitamin D deficiency in BC cases in the twin cities.

Patients and Methods

The case-control study was conducted at the Department of Pathology, Pak-Emirates Military Hospital, Rawalpindi, Pakistan from February 5 to December 12, 2024, after approval from the institutional ethics review board. The sample size was calculated using the World Health Organisation (WHO) calculator based on data reported in literature.[9] The sample was raised using non-probability consecutive sampling technique. Those included were females based in Rawalpindi and Islamabad who were aged 20-75 years and were diagnosed with invasive BC classified as stage III with in the preceding six months. They were placed in group A. Age-matched healthy females without any breast pathology were enrolled in control group B. Written informed consent was taken from all the participants in both the groups. Those excluded were male patients, pregnant or lactating females, those with stage IV BC, and patients receiving neoadjuvant chemotherapy (NAC). Also excluded were patients undergoing dialysis, having elevated liver enzymes, primary hyper-parathyroidism, hypercalcaemia, hypercalciuria, or Paget's disease, and those already taking therapy to improve vitamin D levels.

Sociodemographic data of the participants was recorded on a pre-designed proforma. The patients were classified as postmenopausal if they had not had menstrual periods for a minimum of 12 months. The histopathology reports of group A patients were used to document the BC grade and stage. A venous sample (4ml) of 25(OH)Dwas collected in a -topped lithium (Li) Li-heparin tube. The serum was separated within one hour after collection by centrifugation at 4000 rpm. Further analysis was done using commercial kits (Elecsys Vitamin D total III Roche Diagnostic, Germany) to measure total 25-hydroxyvitamin D in serum/plasma on cobas analyses. The levels of 25(OH)D were measured using electro-chemiluminescence immunoassay on an analyser (Roche COBAS e411 Roche Diagnostics, Germany) by using specific Roche reagents, running regular 2-point calibrations, maintaining 2–8°C storage for reagents, ensuring proper sample pre-treatment, and the values were documented in nmol/L terms. Vitamin D assay had a sensitivity of 96% and specificity of 94%. Vitamin D levels were considered deficient at serum level <25nmol/L, insufficient at 25-75nmol/L and sufficient at 75-250nmol/L.[11]

Data was analysed using SPSS21. Categorical variables were presented as frequencies and percentages, while mean +/- standard deviation values were calculated for numerical variables. Kolmogorov-Smirnov test was used to check data normality. A chi-square analysis was done for categorical variables, and Mann-Whitney test for numerical variables. P≤0.05 was considered statistically significant.

Results

Of the142female subjects, 71(50%) were in group A with mean age 46.9±16.3 years, and 71(50%) were in group B with mean age 42.4±15.9 years (p=0.797). Detailed sociodemographic data was noted for all the subjects in the two groups (Table 1).

Vitamin D levels were also lower in group A patients compared to those in group B (p<0.001). Vitamin D deficiency was observed in 33(46.5%) cases compared to 5(7%) controls, with supplement use being less among the cases 11(15.5%) compared to the controls 28(39.4% (p=0.001). Other characteristics, such as height (p=0.682), weight (p=0.501) and body mass index (BMI) (p=0.528) had no significant difference between the groups (Table 2).

There was a high level of correlation of vitamin D deficiency with positive family history of BC (p<0.001) and use of supplements (p=0.001) (Table 3).

Discussion

Vitamin D deficiency is highly prevalent in Southeast Asia, with a reported prevalence ranging from 70% to 97% among healthy, asymptomatic individuals in Pakistan alone. This deficiency is more commonly observed in urban populations.[12] The incidence of deficiency of vitamin D levels in Southeast Asia is approximately double of that observed in Western populations.[13] Postmenopausal females having osteoporosis are particularly prone to the deficiency of vitamin D.[14] In one study done in the United States, 50-74% of freshly diagnosed premenopausal BC

Table-1: Baseline characteristics of the participants.

Study Variables	n (%)
Study Groups	
Case	71 (50.0)
Control	71 (50.0)
BMI Group	
Underweight	2 (1.4)
Normal	52 (36.6)
Overweight	81 (57.0)
Obese	7 (4.9)
Marital Status	
Married	133 (93.7)
Unmarried	9 (6.3)
Parity	
≤2	39 (27.5)
≥3	103 (72.5)
Menopausal Status	
Nil	15 (10.6)
Regular Stopped	66 (46.5)
Stopped	61 (43.0)
Vitamin D Supplement	
Yes	39 (27.5)
No	103 (72.5)
Vitamin D Level	
Deficient<25 nmol/L	38 (26.8)
Insufficient = 25-75 nmol/L	63 (44.4)
Sufficient= 75-250 nmol/L	41 (28.9)
Parda Observing	
Yes	68(47.9)
No	74 (52.1)
Sun Exposure	
<20 Min	67 (47.2)
20-30 Min	60 (42.3)
>30 Min	15 (10.6)
Occupation	
Working	33 (23.2)
Housewife	109 (76.8)
Family Income	
<30,000	22 (15.5)
30,000-50,000	93 (65.5)
>50,000	27 (19.0)
Educational Status	
Illiterate	39 (27.5)
Primary	34 (23.5)
Matric or more	69 (48.5)
Family History of Breast Carcinoma	
Yes	64 (45.1)
No	78 (54.9)

BMI: Body mass index.

females exhibited vitamin D deficiency.[15]

The current findings suggested that 86.8%% of BC females and 13.2% of healthy females exhibited vitamin D deficiency. This is comparable to a study where levels of serum 25(OH) D in BC women were markedly lower compared to healthy Pakistani women.[16]

Table-2: Intergroup comparison of continuous variables.

Variables	Study Groups		*p*-value
	Case Median (IQR)	Control Median (IQR)	
Age (years)	50.0 (30.00–64.00)	49.0 (30.00–60.00)	0.797
Height (Feet)	5.40 (5.20–5.50)	5.40 (5.20–5.50)	0.682
Weight (Kg)	70.00 (65.00–75.00)	69.00 (60.00–75.00)	0.501
BMI	26.30 (23.80–28.20)	25.80 (23.80–27.50)	0.528
Vitamin D Level	41.03 (21.59–65.50)	65.50 (51.67–90.00)	<0.001*

*statistical significant; IQR: Interquartile range.

Table-3: Intergroup comparison of correlational values.

Variable	Categories	Study Groups	*p*-value	
		Case n (%)	Control n (%)	
Vitamin D Level	Deficient (<25 nmol/L)	33 (46.5)	5 (7.0)	<0.001
	Insufficient (25–75 nmol/L)	24 (33.8)	39 (54.9)	
	Sufficient (75–250 nmol/L)	14 (19.7)	27 (38.0)	
BMI Group	Underweight	1 (1.4)	1 (1.4)	0.972**
	Normal	25 (35.2)	27 (38.0)	
	Overweight	41 (57.7)	40 (56.3)	
	Obese	4 (5.6)	3 (4.2)	
Marital Status	Married	66 (93.0)	67 (94.4)	0.500
	Unmarried	5 (7.0)	4 (5.6)	
Parity	≤2	21 (29.6)	18 (25.4)	0.354
	≥3	50 (70.4)	53 (74.6)	
Menopausal Status	Nil	7 (9.9)	8 (11.3)	0.872
	Regular	32 (45.1)	34 (47.9)	
	Stopped	32 (45.1)	29 (40.8)	
Vitamin D Supplement	Yes	11 (15.5)	28 (39.4)	0.001
	No	60 (84.5)	43 (60.6)	
Parda Observing	Yes	31 (43.7)	37 (52.1)	0.201
	No	40 (56.3)	34 (47.9)	
Sun Exposure	<20 Min	36 (50.7)	31 (43.7)	0.702
	20–30 Min	28 (39.4)	32 (45.1)	
	>30 Min	7 (9.9)	8 (11.3)	
Occupation	Working	19 (26.8)	14 (19.7)	0.214
	Housewife	52 (73.2)	57 (80.3)	
Family Income	<30,000	13 (18.3)	9 (12.7)	0.283
	30,000–50,000	42 (59.2)	51 (71.8)	
	>50,000	16 (22.5)	11 (15.5)	
Educational Status	Illiterate	22 (31.0)	17 (23.9)	0.641
	Primary	16 (22.5)	18 (25.4)	
	Matric or more	33 (46.5)	36 (50.7)	
Family History of Breast Carcinoma	Yes	43 (60.6)	21 (29.6)	<0.001*
	No	28 (39.4)	50 (70.4)	

** Fisher's Exact Test applied due to small expected counts in some categories;
* Statistical Significant; BMI: Body mass index.

Imtiaz S et al. in 2012 suggested that the variables, such as marital status, age, adherence to 'parda', BMI, residential area and menopausal status, were evenly distributed among both cases and controls.[17] Vitamin D deficiency was observed in 77% of the control group, and 95.6% of the cases (p<0.001). This is comparable to the current study.

Amjad A et al. in 2023 reported that the mean age for cases was 47.8±11.6 years, while for the controls it was 33.7±12.7 years. In the current study, the mean ages for cases and

controls were 46.92±16.32 years and 42.39±15.95 years, respectively. The mean BMI for the controls was 26.6±3.4kg/m^2 compared to 27.9±3.3kg/m^2 for the cases. Other biological variables did not show significant variation between the groups. In contrast to the current study, other biological variables, along with BMI, did not exhibit substantial differences between the categories. The adjusted odds ratio (AOR) for cases in the study indicated a significant association between low vitamin D levels and higher BMIs with higher BC risk.[18] These results were in line with the current study except for the association of higher BMI with increased BC rate. Additionally, adjustment for age, use of vitamin D supplements, and sun exposure did not show any remarkable link with the risk of developing BC by Amjad et al.[18] However, in the current study, the lower frequency of vitamin D supplement usage was found to be indicative of high BC risk.

Shamsi U et al. in 2020 found that females with 25(OH)D deficiency (<20ng/mL) had a greater probability of getting BC compared to those having sufficient levels of serum 25(OH)D (>30ng/mL). The present study also indicated that females with vitamin D deficiency have a higher probability of developing BC, but the threshold for vitamin D deficiency was set at <25nmol/L in the present investigation. Furthermore, in line with the current results, the earlier investigation also revealed that females who had taken vitamin D supplements 12 months prior to being enrolled in the study showed an evident protective benefit againstBC.[19]

In addition, the deficiency of vitamin D may weaken immune surveillance, thus letting the abnormal cells grow and develop tumours. The present study's results are in line with the results of a prospective observational research carried out in the Mediterranean region by Buono G et al. in 2017[20] with respect to correlation between lower blood 25(OH)D concentrations and BC. WIn the earlier study, deficient vitamin D levels were found to be associated with high-grade BC, and node-positive cases.[20] A case-control investigation in China in 2013 along with a meta-analysis of 21 independent studies also suggested that vitamin D may exert a chemo-preventive effect against BC.[21]

Vitamin D is an immune-modulating anti-inflammatory anti-proliferative hormone. It suppresses growth of tumour cells, and stimulates cell differentiation and apoptosis. The gene control of the cell cycle is controlled by the activation of VDR, which is present in the breast tissue. VDR gene polymorphisms, including FokI and TaqI variants, have been reported to modify receptor sensitivity and determine personal susceptibility to BC.[22] The current results demonstrate the relevance of vitamin D screening and supplementation in women, especially those who are at BC risk. Given the deficiency prevalence in Pakistan, some of the most basic levels of prevention may include fortification of food, encouraging sunlight exposure, and regular vitamin D testing. Supplementation has been found to lower the BC risk and can also improve the prognosis when it is combined in the normal treatment regimens.[10,19]

The current study has limitations as it was done at a single centre over a short duration with a small sample size. The small sample constrained the capacity to detect statistically significant trends between vitamin D deficiency and the histopathological features of BC. Serum 25(OH)D levels indicated recent vitamin D intake rather than long-term exposure. Consequently, one measurement of serum 25(OH)D in the study may not accurately reflect vitamin D status for an extended period. Multicentre studies over larger time spans and with comparatively large sample sizes are recommended.

Conclusion

There was a correlation of vitamin D deficiency with an increased BC risk, while preventive effects of vitamin D supplement against BC were documented. Sufficient vitamin D supplementation in the female population may play a vital role in reducing the incidence of BC in the local population.

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UF, UN, QAM, SB, AS & SBB: Concept, design, data acquisition, analysis, interpretation, drafting, revision, final approval and agreement to be accountable for all aspects of the work.