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Serum Vitamin D in Women with Pre and Post Menopausal Newly Diagnosed Breast Cancer in Pakistan

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Abstract A large population of Pakistan is suffering from vitamin D deficiency. Decreased levels of vitamin D are associated with many critical diseases. In Pakistan, breast cancer incidence is on the rise especially among young women. Vitamin D anti-carcinogenic effects are known to protect from breast cancer. This study determined serum vitamin D level in newly diagnosed breast cancer and healthy premenopausal and postmenopausal women and to evaluate its association with age groups, menopausal status, body mass index, grade, stage, size of tumor and serum calcium level. The study included 300 newly diagnosed breast cancer women visiting INMOL and 300 age-matched healthy women from different areas of Punjab province. Blood samples were collected and serum vitamin D and calcium levels were estimated. The mean±SD vitamin D levels in patients and control group were 11.4±6.1and 23.8±5.8ng/ml respectively (p<0.001). Pre and postmenopausal breast cancer women had mean vitamin D levels 10.6±4.2 and 12.6±7.5ng/ml respectively (p<0.001). No significant association of tumor grade and stage whereas a significant association (p<0.001) of tumor size with low vitamin D levels among breast cancer patients was found. A significant association was also found between low vitamin D and high calcium levels among pre and post menopausal breast cancer women (p<0.001) and high calcium levels with increased tumor size p<0.001. The study showed severe vitamin D deficiency in breast cancer women which must be appropriately managed because it may lead to tumor aggressiveness, poor prognosis of disease and other related complications.

Keywords: *breast cancer, menopausal status, tumor stage and size, vitamin d deficiency, calcium status*

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1. Introduction

During last decade, Vitamin D has emerged to have important role in risk stratification to various cancers. It has not only important role in calcium homeostasis, but has numerous immunologic and antiproliferative activities in the human body [1,2]. Vitamin D has anticarcinogenic effects and found to protect from breast cancer by binding to vitamin D receptor (VDR) [3]. Vitamin D is synthesized in skin from 7-dehydrocholesterol to precalciferol which subsequently isomerizes to cholecalciferol (Vitamin D). Vitamin D is hydroxylated in liver to 25-hydroxyvitamin D which then transported to kidney and is converted to 1,25-dihydroxyvitamin D (Calcitriol). This active form of vitamin D binds to vitamin D receptors (VDRs) and leads to specific gene expression [4]. The concentration of 25(OH) vitamin D_3 serves as a biomarker for the vitamin D status of individuals [5]. VDR is found in almost all tissues and organs of the human body, and is active in the transcription of numerous genes responsible for cell-cycle control, apoptosis, and metastatic potential [3,6]. In breast cells, polymorphism of VDR genes has been postulated to increase both the risk of occurrence and prognosis of breast cancer [7]. Vitamin D deficiency and insufficiency is common in breast cancer patients and is associated with poor prognosis in breast cancer [8,9,10,11].

Intake of vitamin D >800IU/day is associated with a small decrease in risk of breast cancer among postmenopausal women [12]. Vitamin D levels are lower in overweight breast cancer women and with high grade tumors. Its level is also decreased in females receiving adjuvant chemotherapy as compared with young and newly diagnosed patients [10]. Vitamin D level decreases with increasing BMI and are higher in peri- and postmenopausal women as compared with premenopausal breast cancer patients [10]. Hence by improving vitamin D levels in females there is less chance to develop breast cancer [13]. Vitamin D also helps in transport of calcium and phosphorous in intestines for bone mineralization. Vitamin D and calcium both are necessary to maintain bone density and an independent risk factor for falls among the elderly [14]. Experimental, epidemiological and clinical studies suggest that calcium and its regulating hormones like vitamin D affect breast cancer risk [15]. Vitamin D_3 level is lower and calcium level is high in breast cancer women with the aggressiveness of disease and it also leads to further complications like osteomalacia and osteoporosis [15]. In United States several retrospective and prospective studies that evaluated circulating concentrations of 25(OH)D, support the concept that vitamin D deficiency increases

the risk of developing osteoporosis and dying from cancer [16].

Prevalence of vitamin D deficiency (VDD) of 92% and 81% in ambulatory patients has also been reported from Pakistan's different diagnostic centers in Karachi and Lahore respectively [17]. Furthermore, studies from Tunisia, New Zealand and the United States reported insufficient vitamin D status in 47.6%, 54% and 100% of their participants respectively [18].

Extensive studies about vitamin D levels in general asymptomatic population of Pakistan are available in the literature. The prevalence of vitamin D deficiency in Pakistani population has been reported in the range of 70-97% in Pakistan and this is more common in the urban population [14]. However, few studies are done that investigate any association between breast cancer risk and serum levels of vitamin-D in Pakistan. The rationale of conducting this study is based on two observations a) there is dearth of published data on prevalence of vitamin-D deficiency in breast cancer from South East Asia b) a high incidence of breast cancer in Pakistan. 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 with grade, stage of the tumor and other factors.

2. Methodology

2.1. Study Design and Data Collection

The study design was case-control and was carried out at Institute of Nuclear Medicine and Oncology Lahore (INMOL) from June 2014 to June 2016. Three hundred newly diagnosed breast cancer and 300 age-matched healthy women (controls) were included in this study. The control women were from different areas of Punjab province and were not relatives. These women had no history of any personal malignancy as well as no family history of breast cancer. Moreover, the control group was not taking any calcium or vitamin D supplements. The control group ages ranged from 19 to 75 years, with an average age of 44 years. The breast cancer patients were recruited from INMOL Cancer Hospital and Breast Clinic of Sir Ganga Ram hospital, Lahore. Both hospitals are tertiary care hospitals where patients come from different areas of Punjab province. The age ranges from 19-75 with an average age of 44 years. Details of tumor size, stage, lymph node status, histological grade, hormone receptor status (ER/PR+, Her2+, ER/PR+, Her2-, ER/PR-, Her2+, ER/PR-, Her2-) were collected from medical records and pathology reports. Personal information such as age, age at menarche and menopause, residential status, number of children (Parity) and gravidity, occupation and physical activity was obtained by direct questioning from the study population. Kolmogorov smirnov test was applied to check the normality of distribution in studied population. The two groups were sampled from populations with different distributions (one normal population taken as control and other breast cancer patients) as determined by the $p<0.05$. Levene's test was applied to check equality of variance and $p> 0.05$ indicated that the variability in two conditions was about the same. All the participants agreed to participate and a written informed consent was taken from each participant of the study. Approval of the study was obtained from scientific research review and ethical committee (registration no. 11-PhD/LCWU-20244) of the institution. Blood samples (5ml each) were collected by venipuncture by disposable syringes. Serum vitamin D levels and calcium levels were estimated by commercially available Enzyme Linked Immunosorbent Assay (ELISA) kit (Immunotech a Beckman Coulter Company, France) and colorimetric kit (Chema, Italy) respectively. The values of vitamin D levels were represented in ng/ml and calcium were represented in mg/dl. The criteria for interpretation of serum vitamin D levels are: Vitamin D deficiency: < 20 ng/mL (50 nmol/L), Vitamin D insufficiency: 21 to 29 ng/mL (50 to 75 nmol/L), Normal level of vitamin D: ≥ 30 ng/mL (75 nmol/L), Vitamin D intoxication: > 150 ng/ml (375nmol/L) ([16,19]).

2.2. Statistical Analysis

Data analysis was done by using SPSS version 20. The demographic variables and the descriptive measures in pre and post menopausal controls and patients were presented in percentages, frequencies and mean±SD. Normality of distribution and equality of variance was determined by kolmogorov smirnov and Levene's tests respectively. Vitamin D level and its association with stages and grade of tumor was determined by chi-square test where as its association with tumor size and calcium levels was determined by t-test. Comparison of vitamin D levels between pre- and postmenopausal status was done by using a *t*-test. A p value ≤0.05 was considered statistically significant.

Table 1. Characteristic features of breast cancer and healthy women

Subjects Characteristics	Breast cancer patients (mean±SD)	Healthy controls (mean±SD)
Age (years)	44.0±10.9	44.1±19.1
Age at menarche (years)	12.8± 0.6	12.8±0.7
BMI (kg/m^2)	25.6±4.5	24.1±4.9
Marital status	Percentage & Frequency	
Married	96 (n=288)	92 (n=276)
Unmarried	4 (n=12)	8 (n=24)
Nulligravida	11 (n=34)	19 (n=58)
Multiparous	89 (266)	81 (n=242)
Physical activity		
Low	70 (n=210)	66 (n=198)
moderate	27 (n=82)	31 (n=94)
High	3 (n=8)	3 (n=8)
Habitat		
Urban	65% (n=196)	69% (n=208)
Rural	35% (n=104)	31% (n=92)
Educational status		
Literate	75% (n=226)	56% (n=168)
Illiterate	25% (n=74)	44% (n=132)
Occupational status		
Housewives	94% (n=282)	65%(n=194)
Working	6% (n=18)	35% (n=106)
Menopausal status		
Premenopausal	55% (n=166)	45% (n=136)
Postmenopausal	45% (n= 134)	55% (n=164)

3. Results

The mean±SD age of breast cancer patients and controls is given in Table 1. Study population was divided into three age groups (young=15-24years, adults 25-64 years and elders 65years- above) based upon age classification criteria. The mean±SD vitamin D levels in three age groups of patients cohort was 6.4±0, 11.3±5.7 and 16.9±12.8ng/ml and control age groups was 25.7±4.8, 22.3±5.8 and 27.8±9.3ng/ml respectively. No significant association (p>0.05) of age with vitamin D level was found in both groups. Marital status, gravida, parity, physical activity, residential status, education, occupation and menopausal status of cases and control women is given in Table 1.

The mean vitamin D level of patients and controls living in urban areas was 11.1±5.7and 25.0±5.1ng/ml as compared to those living in rural areas 12.0±6.8 and 21.1±6.3ng/ml respectively. Breast cancer patients and control women who were housewives had mean vitamin D level 11.4±6.1 and 22.5±6.0ng/ml and working women had 12.0±6.2 and 26.1±5.0ng/ml respectively. Participants with breast cancer and control group with low physical activity had mean vitamin D level 11.2±6.2 and 24.0±6.0ng/ml respectively.

The mean±SD BMI of patient and control group is shown in Table 1. According to BMI classification breast cancer women were 2% underweight, 51% normal, 34% overweight and 13% obese. The mean vitamin D level in patients and control groups with BMI ≥30kg/m^2 (Obese) was found to be 9.2±3.4ng/ml and 23.2±6.4 ng/ml respectively.

Serum vitamin D levels among the participants of the study was found to be lower in patients as compared to control group (Table 2).

According to menopausal status the results (Table 3) clearly indicated that serum vitamin D levels were deficient in pre and postmenopausal breast cancer patients and insufficient in control group (p<0.001).

Patients included in this study had different stages of breast cancer. About 13% (n=40) had stage I with mean vitamin D level 11.1±4.9ng/ml, 47%(n=142) had stage II with mean vitamin D level 11.2±6.3ng/ml, 31%(n=92) had stage III with mean vitamin D level 12.1±6.4ng/ml and 9%(n=26) had stage IV cancer with mean vitamin D level 10.7±5.4ng/ml. Mean vitamin D concentrations with respect to different stages is shown in Figure 1.

Table 2. Serum vitamin D levels in breast cancer patients and healthy controls

Serum vitamin D levels (ng/ml)	Breast cancer patients			Healthy controls			
	Mean±SD	N	%age	Mean±SD	N	%age	p Value
	11.4±6.1			23.8±5.8			p<0.001*
Insufficiency		14	5		184	61	p=0.33**
Deficiency		280	93		80	27	p=0.20**
Sufficiency		6	2		36	12	P=0.23**

* t-test, **chi-square test.

Table 3. Menopausal status of breast cancer and healthy women

Menopausal status	Deficient vitamin D levels	Insufficient vitamin D levels		
Premenopausal breast cancer females	10.6± 4.2ng/ml	Premenopausal healthy women	26.0±5.1ng/ml	p<0.001*
Postmenopausal breast cancer women	12.6±7.5ng/ml	Postmenopausal healthy women	22.0±5.8ng/ml	p<0.001*
P value	p<0.001*		p<0.001*	

* t-test.

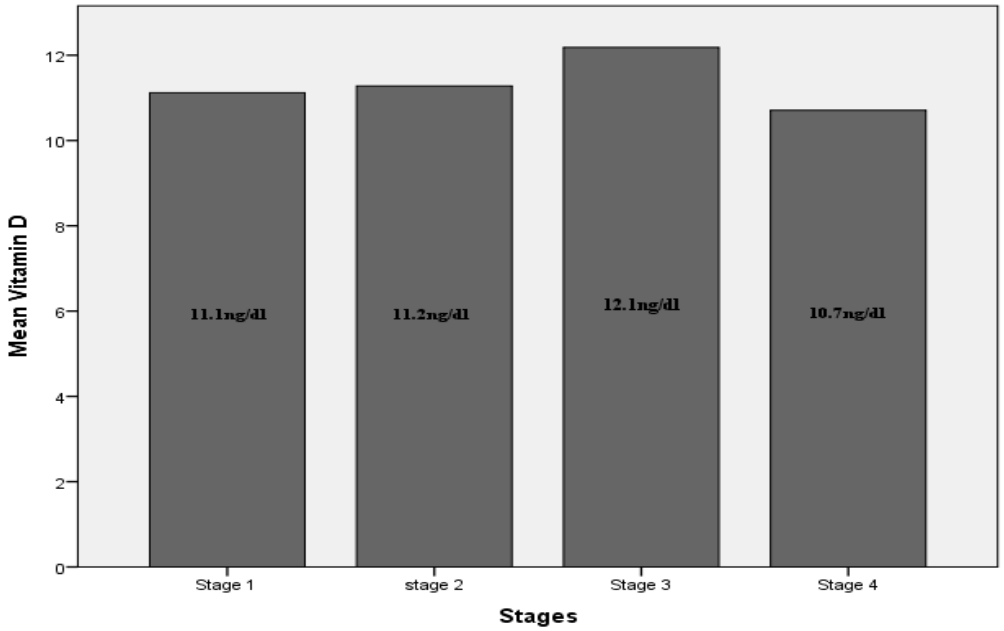

Figure 1. Vitamin D in different stages of breast caner

Table 4. Vitamin D levels in breast cancer patients with different hormonal receptor status

Vitamin D levels Mean	ER/PR+,Her2+	ER/PR+,Her2-	ER/PR-,Her2+	ER/PR-,Her2-	p-value
	10.0±5.9ng/ml	12.9±6.8ng/ml	9.0±2.7ng/ml	12.9±5.9ng/ml	0.01***

***ANOVA test.

This difference in vitamin D level among patients with different grades of disease was not found significant (p=0.81) by ANOVA test.

Patients suffering from ductal carcinoma were 88% (n=264) and 12%(n=36) had lobular carcinoma. Right breast of 50%(n=150) patients was affected and 49% (n=148) patients had cancer in left breast whereas in 1%(n=2) patients both breasts were affected. Axillary lymph node status was positive in 97% (n=290) and negative in 3% (n=10) breast cancer women in present study.

According to receptor status ER/PR+,Her2+ receptor status was found in 35%, in 30% ER/PR+, Her2-, ER/PR-,Her2+ in 12% and ER/PR-,Her2- in 23% breast cancer patients. Breast cancer patients with different hormonal receptor status had deficient mean serum vitamin D level as shown in Table 4.

The difference in vitamin D level in cancer patients with different group of receptor status was significant with p value 0.01 by ANOVA test. A significant difference of vitamin D level was found between patients with receptor status ER/PR+, Her2- and ER/PR-, Her2+ with p value 0.009 and patients with receptor ER/PR-,Her2+ and ER/PR-,Her2- with p value 0.01.

The difference in vitamin D level and tumor size in different stages of breast cancer patients was analyzed by MANOVA and p-value 0.91 showed that vitamin D level and tumor size is not stage dependent whereas a significant difference (p=0.001) of vitamin D level and tumor size was found with receptor status in breast cancer patients in this study.

In present study breast cancer women with mean±SD decreased tumor size (5.3±2.3cm) have increased vitamin D level (11.6±6.2ng/ml) and patients with increased tumor size (17.9±7.6cm) have decreased vitamin D levels (10.9±5.7ng/ml). Results of the study showed no significant association of tumor grade and stage whereas a significant association of tumor size with low serum vitamin D levels among breast cancer patients (p<0.001).

Calcium levels in breast cancer patients and healthy controls were also estimated in this study. The mean±SD serum calcium levels between patients and control groups were 8.7±1.4 and 8.4±0.9mg/dl respectively. In this study a comparison of patients' tumor size with mean serum calcium levels was also done. The breast cancer patients with increased (17.9±7.6cm) and small tumor size (5.3±2.3cm) had mean serum calcium level 9.5±1.5mg/dl and 8.4±1.2mg/dl respectively with p value <0.001. A slight positive correlation (r=0.19) indicated a significant association of increased calcium level with increased tumor size in breast cancer women. The mean calcium level was also high in overweight postmenopausal patients (9.2±2.2mg/dl) as compared with overweight premenopausal (8.6±0.7 mg/dl) breast cancer females. There was a significant association found between low vitamin D and high calcium levels among pre and post menopausal breast cancer females with p value <0.001.

4. Discussion

Vitamin D deficiency is becoming a worldwide problem in general population. Vitamin D deficiency has been found to be associated with a variety of cancers, including prostate, multiple myeloma, colorectal and breast cancer. There are many factors that contribute in its deficiency like reduced exposure to sunlight, age-linked reduction in cutaneous synthesis and intake of food with reduced vitamin D levels [20]. Nationwide studies showed high incidence of vitamin D deficiency (VDD) of 92% and 81% in ambulatory patients from Pakistan's different diagnostic centers in Karachi and Lahore respectively [17]. The present study determined vitamin D levels in pre and post menopausal newly diagnosed breast cancer and healthy females. If healthy premenopausal women are deficient in vitamin D levels then they may have a risk to develop breast cancer and other related complications in their later age. Whereas in breast cancer women vitamin D deficiency is a contributing factor of tumor aggressiveness and poor prognosis of a disease. Our population comprised mainly middle age with almost equal distribution of pre- and postmenopausal females from different areas of Punjab province. Vitamin D deficiency in healthy asymptomatic people is reported in the range of 70-97% and this is more common in the urban population [17]. The incidence is twice that in western population. In newly diagnosed premenopausal breast cancer patients, vitamin D deficiency of 50-70% is reported from United States [15]. The results of the current study showed high prevalence of vitamin D deficiency (93%) in breast cancer women whereas healthy controls (61%) were vitamin D insufficient. Moreover the results for the healthy control group lie within the range reported in the Pakistani literature. Serum levels of vitamin D in breast cancer patients were significantly lower than control Pakistani women. The association between breast cancer risk and vitamin D deficiency was similar to different studies from the developed countries and is a major indicator of poor prognosis in breast cancer patients [11,21].

The results of the current study showed that among the breast cancer patients majority of women (65%) were from urban areas, literate (75%), married (96%) and multiparous (89%) and were vitamin D deficient (11.1±5.7ng/ml) and is expected due to lack of optimal nutrition in this group. Most of the patients (94%) were house wives with low physical activity and had low vitamin D levels (11ng/ml) probably due to minimum sun exposure. This may be due to lack of optimal nutrition in this group and minimum sun exposure. A study on healthy population from urban areas of Pakistan showed 70-97% population vitamin D deficient [17]. Imtiaz *et al* [21]. reported low vitamin D levels in breast cancer women from urban areas who were married, multiparous and housewives. Various other epidemiological studies had reported that lower serum vitamin D levels are associated with obesity and lower physical activity [22,23].

In present study 13% patients with BMI ≥30kg/m^2 were vitamin D deficient (9.2±3.4ng/ml). These results coincide with previous study that showed vitamin D deficiency in 31% obese breast cancer women [21].

The results of the present study showed deficient vitamin D levels (10.6±4.2ng/ml) in newly diagnosed premenopausal breast cancer patients. Khan and Fabian [24] reported 50-74% vitamin D deficiency in newly diagnosed premenopausal breast cancer patients from United States. As vitamin D is responsible for the production of estrone (21%), estradiol (9%) and progesterone (13%) in ovarian tissues of women. It may reduce the risk of breast cancer by regulating the production of these hormones [25].

Vitamin D levels were evaluated in breast cancer patients of stage I, II, III and IV. No significant difference of vitamin D level was found in patients with different stages of breast cancer and all the patients were found to be vitamin D deficient with *p*-value >0.05 by ANOVA test. Ductal carcinoma was more prevalent (88%) in this study as compared to lobular carcinoma (12%) and results were similar to the study conducted by *Hosseini et al.* [26].

In present study patients with ER/PR$^-$,Her2+ receptor status had low mean (9.0±2.7ng/ml) vitamin D levels as compared to patients with receptor status ER/PR+,Her2- (12.9±6.8ng/ml) and ER/PR$^-$,Her2- (12.9±5.9ng/ml) and there was a significant difference of vitamin D level in patients with different receptor status (p= 0.01). Whereas one previous study showed that patient with all types of receptor status had same vitamin D levels of no statistical significance [21]. However Kim *et al.* [11] reported highest percentage of vitamin D deficiency in patients with triple negative receptor status.

Our study showed that vitamin D levels significantly decrease (10.86±5.0ng/ml) with increase in tumor size (p<0.001). The same result of low vitamin D levels with increased tumor size was reported in previous study conducted by Goodwin *et al.* [10]. Previous studies also determined that vitamin D interfere with the transduction pathways of a variety of growth factors-activated receptors (receptor tyrosine kinases) and modulate transcription and inhibition of cell proliferation and angiogenesis that leads to cell differentiation and apoptosis. It also increases the level of an endogenous protein - cystatin D, which have antitumor and antimetastatic property [27].

In present study calcium levels were also estimated as vitamin D contribute in regulation of calcium metabolism and it is a major risk factor of other complications like osteomalacia and osteoporosis in breast cancer patients. The results of the study demonstrated that with the increase of tumor size (17.9±7.6cm) serum calcium level increases (9.5±1.5mg/dl). Previous studies also showed that breast cancer patients with advance stage cancers had hypercalcemia as the size of tumor increases it proemotes calcium leakage from the bones which leads to osteomalacia and osteoporosis ([28,29]). Therefore, an adequate concentration of vitamin D is required to prevent from osteomalacia and osteoporosis because it mobilize calcium from the bone and it also conserve calcium from urine in this way it induces bone resorption [30]. The mean calcium level was also high in postmenopausal, overweight patients (9.2±2.2mg/dl) as compared with premenopausal overweight (8.6±0.7mg/dl) breast cancer females. There was a significant association found between low vitamin D and high calcium levels among pre and post menopausal breast cancer women (p<0.001). Previous studies reported that low vitamin D and high calcium levels are positively associated with worse grade of tumor in breast cancer women [10,15].

5. Conclusion

Vitamin D deficiency and insufficiency is highly prevalent in breast cancer and healthy premenopausal and postmenopausal women respectively. In breast cancer women low vitamin D levels and high calcium levels are significantly associated with increased tumor size which may lead to poor prognosis of disease and other related problems like osteomalacia and osteoporosis. This study diverts the attention of clinicians and oncologists to aware general population and breast cancer women to monitor their vitamin D and calcium levels at least once in six months and recommended to take fortified food, vitamin D and calcium supplements to prevent them from further complications.

Present study has limitations. First, frequency of vitamin D insufficiency appeared to be higher in both groups. Sun exposure and dietary vitamin D intake was not measured explicitly in this study. Assessment of vitamin D status should be part of a regular nutrition assessment for most women. Dietary vitamin D and casual sunlight exposure may be among the modifiable risk factors for breast cancer. Well-designed larger clinical trials are needed to look at the social and dietary associations in breast cancer women with vitamin D deficiency. Also further studies are required to determine role of vitamin D receptor gene polymorphism in healthy and breast cancer women to evaluate whether vitamin D receptor status is associated with the risk of developing breast cancer in Pakistani women population.

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