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STUDY OF SERUM VITAMIN D_3 LEVELS AND RISK OF BREAST CANCER

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Abstract:
Objective: To assess the Risk Associations of Vitamin D_3 levels and Breast Cancer and evaluate the Correlation between Vitamin D_3 levels and Breast Cancer.
Materials and Methods: This was a prospective case-control study of 50 newly diagnosed cases with Breast Cancer. The serum level of 25-hydroxy vitamin D [25(OH)D was measured by ELISA at the time of diagnosis and before the start of cancer treatment. Vitamin D deficiency was defined as 25(OH) D<10 ng/mL and Insufficiency as serum 25(OH)D levels of <20 ng/ml. Patients were followed up for a median of 18 months.
Result: In our study, the mean value of serum Vitamin D3in study group was 21.86 with SD of 31.61.While the mean value in the control group was 23.62 with a SD of 21.13. There was no substantial difference in level of mean between cases and controls leading to a statistically non-significant result (p value-0.744). In the Insufficient category the mean in the study group was 15.87±1.74 while mean in the control group was 21.87±6.80, with a p value of <0.002 thus being statistically significant. This shows the insufficiency of Vitamin D3was higher in breast cancer cases as compared to controls. On further stratification of subjects into each sub-category depending on Serum Vitamin D3 , 23 cases were found in deficient category,17 cases were in the insufficient category and 10 cases were in sufficient category further stressing upon the fact that Vitamin D deficiency is a risk factor for Breast Cancer.
Conclusion: As specified in above results 40 patients out of 50 cases were having deficient and insufficient levels of Vitamin D_3 in serum thus leading to the conclusion, that deficiency of Vitamin D_3 promotes carcinogenesis.
keywords: 1,25($OH_{)2}$Vitamin $D_{3,}$Breast Cancer, VDR(Vitamin D Receptor)

Introduction

Breast cancer (BC) is the most common form of malignancy and the second leading cause of cancer mortality in women. It is estimated that 1 in 8 women will develop Breast Cancer in their lifetime.[1,2] There are substantial preclinical and epidemiologic data that suggest that vitamin D plays a role in the prevention and treatment of cancer. Numerous observational studies have shown that low blood levels of 25(OH) vitamin D3 (cholecalciferol), estimated by geographical location, diet and activity assessment or measured serum levels are associated with a higher risk of cancer and worse cancer-specific survival as well as numerous morbidities to e.g. cardiovascular disease, stroke, infection, autoimmune disease, and neuromuscular dysfunction among large populations. A considerable number of in vitro and in vivo studies indicate that the most active metabolite of vitamin D – 1,25-dihydroxycholecalciferol or calcitriol – has anti-proliferative, pro-apoptotic, pro-differentiating, and anti-angiogenic properties. Combined treatment with calcitriol and many types of cytotoxic agents has synergistic or atleast additive effects.[3] In our study,we assessed the Risk Associations of Vitamin D_3 levels and Breast cancer and evaluated the Correlation between Vitamin D_3 levels and Breast Cancer.

Material and Methods

The present study was a prospective study including 50 newly diagnosed cases of breast cancer and was carried out in Department of General Surgery in collaboration with Department of Pharmacology, at Guru Nanak Dev Hospital, Government Medical College Amritsar from January 2017 to October 2018 after taking permission from Institutional Ethics Committee, Government Medical College, and Amritsar. Female patients irrespective of age and having confirmed breast cancer by FNAC and Histopathology attending the hospital were the subject of study. Male patients, those with benign breast diseases and those suffering from bowel disease with malabsorption or consuming Vitamin D_3 (within the past two months) were excluded from the study. All the patients meeting the selection criteria underwent routine investigations. The case history of the patient was recorded, including detail history of pain, nipple discharge, ulceration of nipple and duration of lesion. The examination of breast lump was done to record the size and site of lump, consistency, and fixation to skin and underlying tissue, retraction of nipple along with regional lymph node involvement. Consent was

taken after due explanation of the procedure to the patient in their vernacular language.

Methodology:

Patients were divided into 2 groups: 50 breast cancer patients and equal number of age-matched females attending hospital for some other diseases were recruited into the study by consecutive sampling over a period of about two years for this case control study.

Serum $1,25(OH)_2D_3$ levels and serum calcium levels were measured and compared between cases and controls. Each Patient was examined after recording his/her relevant personal, medical and family history thoroughly including physical and systemic examination. All routine Investigations were done. Serum Vitamin D_3 estimation using ELISA technique using kit based on competitive binding.[4] All the collected parameters were tabulated and both groups were compared with each other based on the various outcome variables mentioned above. The comparison groups were evaluated using Student's t-test, Chi Square Test and ANOVA Test.

Results:

Table 1 depicts comparison of mean of serum vitamin D_3levels between Study cases and controls. The mean of Vitamin D_3in study group was 21.86±31.61 while mean of serum vitamin D_3in control group was 23.62±21.13. The p value was 0.744 which was non significant statistically.

Table 1: Comparison of mean of vitamin D3 between group A (cases) and group B (controls).

	Mean	SD	p-value
Group A	21.86	31.61	0.744
Group B	23.62	21.13	
Total	22.74	26.76	

Table 2 predicts the stratification of cases and controls according to levels Vitamin D_3 in serum. The number of cases with deficiency in the study group were 23(46%) while the no cases with deficiency in the control group were 20(40%). The number of cases with insufficient levels of vitamin D_3in serum were 17(34%) in the study group and 16(32%) in the control group. While the number of cases with sufficient levels in the study group were 10(20%) and in the control group the number of cases were 14 (28%).

Table 2: Distribution of cases and control according to severity of vitamin D3 deficiency

Serum Vitamin D3(ng/ml)	Group A		Group B		Total	
	No.	%	No.	%	No.	%
Deficiency (<10)	23	46.0	20	40.0	43	43.0
Insufficiency (10-30)	17	34.0	16	32.0	33	33.0
Sufficient (>30)	10	20.0	14	28.0	24	24.0
Total	50	100.0	50	100.0	100	100.0

Table 3 depicts that mean of vitamin D_3in the Case group in the deficient category is 4.73±3.44 while in control group it is 4.80±3.20. The p value was 0.953 which was insignificant. While the level of Vitamin D_3in insufficient category in the case group was 15.87±1.74 and in the control group was 21.87±6.80. The p value is 0.002 which is significant statistically. In the sufficient category the mean of Vitamin D_3in case group 66.56±44.20 while in the control group 52.50±12.97. The p value was 0.275 thus not significant statistically.

Table 3: Means of vitamin D3 and levels of deficiency

Serum Vitamin D3(ng/ml)	Group A		Group B		r-value	p-value
	Mean	SD	Mean	SD		
Deficiency (<10)	4.73	3.44	4.80	3.20	0.009	0.953
Insufficiency (10-30)	15.87	1.74	21.87	6.80	0.529	0.002
Sufficient (>30)	66.36	44.20	52.50	12.97	-0.227	0.275

Table 4 shows that in the study group the number of cases with SGOT below 30 were 14(28%), those with SGOT between 31-40 were 29(58%)cases, those with SGOT between 41-50 were 6(12%) and those with SGOT above >50 was 1(2%) in number. In the control group, the number of cases with SGOT below 30 were 35(70%), those with SGOT between 31-40 were 12(24%) cases, those with SGOT between 41-50 were 3(6%) and those with SGOT above >50 were none. The p-value was 0.001 which was highly significant.

Table 4: SGOT comparison between cases and controls

SGOT(IU/L)	Group A		Group B		Total	
	No.	%	No.	%	No.	%
<30	14	28.00	35	70.00	49	49.00
31-40	29	58.00	12	24.00	41	41.00
41-50	6	12.00	3	6.00	9	9.00
>50	1	2.00	0	0.00	1	1.00
Total	50	100.00	50	100.00	100	100.00
Mean	34.50±7.08		29.30±5.04		31.80±6.61	
t-value	4.066					
p-value	0.001					

Table 5 shows that in the study group the number of cases with SGPT below 30 were 16(32%), those with SGPT between 31-40 were 31(62%)cases, those with SGPT between 41-50 were 3(6%). In the control group, the number of cases with SGPT below 30 was 32(64%), those with SGPT between 31-40 were 15(30%) cases, and those with SGPT between 41-50 were 3 (6%). The p-value was 0.001 which was highly significant.

Table 5: SGPT comparison between cases and controls

SGPT(IU/L)	Group A		Group B		Total	
	No.	%	No.	%	No.	%
<30	16	32.00	32	64.00	48	48.00
31-40	31	62.00	15	30.00	46	46.00
41-50	3	6.00	3	6.00	6	6.00
Total	50	100.00	50	100.00	100	100.00
Mean	33.40±5.78		29.86±5.04		31.80±6.61	
t-value	3.345					
p-value	0.001					

Discussion

The mean value of serum Vitamin D_3in study group was 21.86 with SD of 31.61.While the mean value in the control group was 23.62 with a SD of 21.13. There was no substantial difference in level of mean between cases and controls leading to a statistically non-significant result (p value-0.744).On further stratification according to levels of vitamin D_3in the blood group, 23(46%) cases were found in deficient category (<10ng/ml) as compared to 20(40%) cases in control group, 17 (34%)cases were with the blood vitamin D_3levels in the insufficient range (10-30 ng/ml)as compared to 16 controls (32%) and only 10 (20 %)cases were in the sufficient range (>30 ng/ml) as compared to 14 (28%)controls.

On comparison of mean between cases and controls in stratified classes of Vitamin D_3there was a significant result in the insufficient category,in which the mean in the study group was 15.87±1.74 while mean in the control group was 21.87±6.80, with a p value of <0.002 thus being statistically significant. This shows the insufficiency of Vitamin $D_3$was higher in breast cancer cases as compared to controls. Thus, the Results of our study were Consistent with a number of studies during yesteryears which proved that there was high level of Vitamin D deficiency and insufficiency in breast cancer cases..One of the recent studies with evidence regarding this is that by ManarAtoum and FoadAlzoughool at Auckland which shows us that most of the vitamin D studies support the inverse association between vitamin D level and breast cancer riskand several retrospective and prospective epidemiologic studies revealed that vitamin D deficiency is associated with increased breast cancer risk.[5]

Another study which was a pooled analysis strongly supported the Inverse relationship between serum 25(OH) Vitamin $D_3$with Breast Cancer. The study concluded that in the estimation of the dose–response gradient, a serum 25(OH)D level of >47 ng/ml would be associated with 50% lower risk of breast cancer, compared to serum 25(OH)D <9.7 ng/ml.[6]

The sunshine vitamin has been associated with reduced risk for many chronic illnesses.Thevitamin D metabolite, 1,25-$(OH)_2$ D_3, has been demonstrated to markedly reduce cellular proliferation especially of malignant cells that have a vitamin D receptor[7].Maalmi et al, reported a meta-analysis of prospective cohort studies and concluded that among breast cancer patients pooled hazard ratios (95% confidence intervals) comparing highest and lowest of 1,25$(OH)_2D_3$ were 0.62 (0.49-0.78) and 0.58 (0.38-0.84) respectively. It was concluded that a 1,25$(OH)_2D_3$ > 30 ng/mL was associated with a significantly reduced mortality in patients with breast cancer.[8]Over 30 years ago, the recognition that VDR expression was retained in breast cancers prompted extensive studies to determine whether targeting VDR in tumors would provide therapeutic benefit. VDR expression is retained in the majority of rodent breast tumors, human breast cancers and established breast cancer cell lines.[9-14]In a study of 136 patients with primary breast cancer, it was found that women with VDR negative tumors relapsed significantly earlier than women with VDR positive tumors.[15]

A recent study demonstrated an association between vitamin D deficiency and poor overall and disease free survival in breast cancer patients. Vitamin D deficiency is positively associated with larger tumors ($p < 0.001$), higher grade ($p = 0.014$), advanced stage ($p = 0.001$), lymph node positivity ($p = 0.012$) and HER2/neu receptor expression ($p = 0.002$). On multivariate analysis, Disease Free Survival,was independently affected by vitamin D deficiency and advanced stage.[16] In our study, serum level of Hb was <8 g/dl in 3 cases (6%), while serum level was between 8 to 10 gm/dl in 31 cases(62%) and >10 g/dl in 16 (32%)cases. The mean value in case group was 8.44±2.80. The results of the study was consistent with a study conducted in GMC-Sector 32, Chandigarh in the department of Community Medicine in the year 2017 which showed high prevalence of hemoglobin deficiency in patients with breast and cervical cancer.[17]While in the control group only 1 case(2%) was present with haemoglobin levels less than 8 gm/dl, while 26 cases(52%) were present with haemoglobin level between 8 to 10 gm/dl and 23 patients (46%)were present with haemoglobin level >10 gm/dl. The mean value in the control group was 10.26±±1.229. There was a significant difference in Haemoglobin values between case and control group hence highly significant statistically (p value-0.001). The mean of Vitamin D between cases and controls in each sub-group of Haemoglobin was also almost similar, with no significant difference thus statistically non significant (p-value->0.05 in each case).

The mean value of SGOT in study group was 34.50±7.08.This results were close to result of study done at PGIMS Rohtak in the department of Genetics and department of biochemistry where the mean value of serum sgot was 27.3±4.02U/L and it raised above 40IU/L after various cycles of chemotherapy.[18]While the mean value in the control group was 29.30±5.04. There was substantial difference in level of mean between cases and controls leading to a statistically significant result ($p=0.001$). The mean of Vitamin D_3 between each sub-group of SGOT between cases and controls was also almost similar, with no significant difference thus statistically non significant (p-value>0.05 in each case).

The mean value of SGPT in study group was 33.40±5.78.This results were close to result of study done at PGIMS, Rohtak in the department of Genetics and

Department of Biochemistry where the mean value of serum SGPT was 27.9±10.24U/L and it raised above 40IU/L after various cycles of chemotherapy.[18]While the mean value in the control group was 29.86±5.04. There was substantial difference in level of mean of between cases and controls leading to a statistically significant result (p=0.001). The mean of Vitamin D_3 between each sub-group of SGPT between cases and controls was also almost similar, with no significant difference thus statistically non significant (p-value>0.05 in each case).The higher mean value of SGOT and SGPT in cases in our study could indicate liver involvement by metastatic disease in case of breast carcinoma patients even early during course of disease as we recruited newly diagnosed cases of breast carcinoma in the study arm. In our study the mean value of serum calcium in study group was 8.21±0.73.While the mean value in the control group was 8.44±0.87. The results were similar to the study, where the mean serum levels in the study group were 8.8 mg/dl and the control group were also 8.8 mg/dl with the p value of 0.932.[19]This Indicated that Vitamin D_3deficiency did not significantly affect the serum calcium levels in cases and no positive correlation was found between Serum Calcium Levels and Breast Cancer

Conclusion

Vitamin $D_3$is known to prevent carcinogenesis, because vitamin D_3has an antiproliferative, antiangiogenic and pro-diferentiating properties. In our study, the mean of vitamin D_3levels between cases and controls was similar. The p-value was 0.744, thus was statistically non-significant.But only 10 cases of breast cancer were with Vitamin D_3levels in the sufficient range above 30ng/ml, indicating that maximum cases had an Insufficient and Deficient level of Vitamin D_3. Also amongst the Insufficient range (10-30ng/ml) of Vitamin D_3there was a substantial difference in the level of mean between cases which was 15.87±1.74 and in controls which was 21.87±6.80 with a p value of 0.002, thus being statistically significant indicating that breast cancer patients had an insufficient levels of Vitamin D_3as compared to controls.

A better understanding is needed in this regard through further studies and will help us develop newer therapeutic modalities like Calcitriol Analogues which will help us to better treat cancers like Breast cancer. Hence more studies are warranted in this regard because of smaller sample size in our study.

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